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(54) Title: MODIFIED TIE-2-RECEPTOR LIGANDS			
(57) Abstract			
<p>The present invention provides for a modified TIE-2 ligand which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. The invention further provides for a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first. In a specific embodiment, the invention further provides for a chimeric TIE ligand comprising at least a portion of TIE-2 Ligand-1 and a portion of TIE-2 Ligand-2. In addition the present invention provides for isolated nucleic acid molecule encoding the modified TIE-2 ligands described. The invention also provides for therapeutic compositions as well as a method of blocking blood vessel growth, a method of promoting neovascularization, a method of promoting the growth or differentiation of a cell expressing the TIE receptor, a method of blocking the growth or differentiation of a cell expressing the TIE receptor and a method of attenuating or preventing tumor growth in a human.</p>			

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MODIFIED TIE-2-RECEPTOR LIGANDS

This application claims the priority of U.S. Serial No. 08/740,223 filed October 25, 1996 and of U.S. Provisional application 60/022,999 filed 5 August 2, 1996. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

INTRODUCTION

10

The present invention relates generally to the field of genetic engineering and more particularly to genes for receptor tyrosine kinases and their cognate ligands, their insertion into recombinant DNA vectors, and the production of the encoded proteins in recipient 15 strains of microorganisms and recipient eukaryotic cells. More specifically, the present invention is directed to a novel modified TIE-2 ligand that binds the TIE-2 receptor, as well as to methods of making and using the modified ligand. The invention further provides a nucleic acid sequence encoding the modified ligand, and methods for 20 the generation of nucleic acid encoding the modified ligand and the gene product. The modified TIE-2 ligand, as well as nucleic acid encoding it, may be useful in the diagnosis and treatment of certain diseases involving endothelial cells and associated TIE receptors, such as neoplastic diseases involving tumor angiogenesis, wound healing, 25 thromboembolic diseases, atherosclerosis and inflammatory diseases. In addition, the modified ligand may be used to promote the proliferation and/or differentiation of hematopoietic stem cells.

More generally, the receptor activating modified TIE-2 ligands described herein may be used to promote the growth, survival, migration, and/or differentiation and/or stabilization or destabilization of cells expressing TIE receptor. Biologically active 5 modified TIE-2 ligand may be used for the in vitro maintenance of TIE receptor expressing cells in culture. Cells and tissues expressing TIE receptor include, for example, cardiac and vascular endothelial cells, lens epithelium and heart epicardium and early hematopoietic cells. Alternatively, such human ligand may be used to support cells which 10 are engineered to express TIE receptor. Further, modified TIE-2 ligand and its cognate receptor may be used in assay systems to identify further agonists or antagonists of the receptor.

15

BACKGROUND OF THE INVENTION

The cellular behavior responsible for the development, maintenance, and repair of differentiated cells and tissues is regulated, in large part, by intercellular signals conveyed via growth factors and similar 20 ligands and their receptors. The receptors are located on the cell surface of responding cells and they bind peptides or polypeptides known as growth factors as well as other hormone-like ligands. The results of this interaction are rapid biochemical changes in the responding cells, as well as a rapid and a long-term readjustment of 25 cellular gene expression. Several receptors associated with various cell surfaces may bind specific growth factors.

The phosphorylation of tyrosine residues in proteins by tyrosine kinases is one of the key modes by which signals are transduced across

the plasma membrane. Several currently known protein tyrosine kinase genes encode transmembrane receptors for polypeptide growth factors and hormones such as epidermal growth factor (EGF), insulin, insulin-like growth factor-I (IGF-I), platelet derived growth factors (PDGF-A and -B), and fibroblast growth factors (FGFs). (Heldin et al., Cell Regulation, 1: 555-566 (1990); Ullrich, et al., Cell, 61: 243-54 (1990)). In each instance, these growth factors exert their action by binding to the extracellular portion of their cognate receptors, which leads to activation of the intrinsic tyrosine kinase present on the cytoplasmic portion of the receptor. Growth factor receptors of endothelial cells are of particular interest due to the possible involvement of growth factors in several important physiological and pathological processes, such as vasculogenesis, angiogenesis, atherosclerosis, and inflammatory diseases. (Folkman, et al. Science, 235: 442-447 (1987)). Also, the receptors of several hematopoietic growth factors are tyrosine kinases; these include c-fms, which is the colony stimulating factor 1 receptor, Sherr, et al., Cell, 41: 665-676 (1985), and c-kit, a primitive hematopoietic growth factor receptor reported in Huang, et al., Cell, 63: 225-33 (1990).

The receptor tyrosine kinases have been divided into evolutionary subfamilies based on the characteristic structure of their ectodomains. (Ullrich, et al. Cell, 61: 243-54 (1990)). Such subfamilies include, EGF receptor-like kinase (subclass I) and insulin receptor-like kinase (subclass II), each of which contains repeated homologous cysteine-rich sequences in their extracellular domains. A single cysteine-rich region is also found in the extracellular domains of the eph-like kinases. Hirai, et al., Science, 238: 1717-1720 (1987); Lindberg, et al. Mol. Cell. Biol., 10: 6316-24 (1990); Lhotak, et al., Mol.

Cell. Biol. 11: 2496-2502 (1991). PDGF receptors as well as c-fms and c-kit receptor tyrosine kinases may be grouped into subclass III; while the FGF receptors form subclass IV. Typical for the members of both of these subclasses are extracellular folding units stabilized by intrachain disulfide bonds. These so-called immunoglobulin (Ig)-like folds are found in the proteins of the immunoglobulin superfamily which contains a wide variety of other cell surface receptors having either cell-bound or soluble ligands. Williams, et al., Ann. Rev. Immunol., 6: 381-405 (1988).

10 Receptor tyrosine kinases differ in their specificity and affinity. In general, receptor tyrosine kinases are glycoproteins which consist of (1) an extracellular domain capable of binding the specific growth factor(s); (2) a transmembrane domain which usually is an alpha-helical portion of the protein; (3) a juxtamembrane domain where the 15 receptor may be regulated by, e.g., protein phosphorylation; (4) a tyrosine kinase domain which is the enzymatic component of the receptor; and (5) a carboxyterminal tail which in many receptors is involved in recognition and binding of the substrates for the tyrosine kinase.

20 Processes such as alternative exon splicing and alternative choice of gene promoter or polyadenylation sites have been reported to be capable of producing several distinct polypeptides from the same gene. These polypeptides may or may not contain the various domains listed above. As a consequence, some extracellular domains may be 25 expressed as separate, secreted proteins and some forms of the receptors may lack the tyrosine kinase domain and contain only the extracellular domain inserted in the plasma membrane via the transmembrane domain plus a short carboxyl terminal tail.

A gene encoding an endothelial cell transmembrane tyrosine kinase, originally identified by RT-PCR as an unknown tyrosine kinase-homologous cDNA fragment from human leukemia cells, was described by Partanen, et al., Proc. Natl. Acad. Sci. USA, 87: 8913-8917 (1990).

5 This gene and its encoded protein are called "TIE" which is an abbreviation for "tyrosine kinase with Ig and EGF homology domains." Partanen, et al. Mol. Cell. Biol. 12: 1698-1707 (1992).

It has been reported that tie mRNA is present in all human fetal and mouse embryonic tissues. Upon inspection, tie message has been
10 localized to the cardiac and vascular endothelial cells. Specifically, tie mRNA has been localized to the endothelia of blood vessels and endocardium of 9.5 to 18.5 day old mouse embryos. Enhanced tie expression was shown during neovascularization associated with developing ovarian follicles and granulation tissue in skin wounds.
15 Korhonen, et al. Blood 80: 2548-2555 (1992). Thus the TIEs have been suggested to play a role in angiogenesis, which is important for developing treatments for solid tumors and several other angiogenesis-dependent diseases such as diabetic retinopathy, psoriasis, atherosclerosis and arthritis.

20 Two structurally related rat TIE receptor proteins have been reported to be encoded by distinct genes with related profiles of expression. One gene, termed tie-1, is the rat homolog of human tie. Maisonpierre, et al., Oncogene 8: 1631-1637 (1993). The other gene, tie-2, may be the rat homolog of the murine tek gene, which, like tie,
25 has been reported to be expressed in the mouse exclusively in endothelial cells and their presumptive progenitors. Dumont, et al. Oncogene 8: 1293-1301 (1993). The human homolog of tie-2 is described in Ziegler, U.S. Patent No. 5,447,860 which issued on

September 5, 1995 (wherein it is referred to as "ork"), which is incorporated in its entirety herein.

Both genes were found to be widely expressed in endothelial cells of embryonic and postnatal tissues. Significant levels of tie-2 transcripts were also present in other embryonic cell populations, including lens epithelium, heart epicardium and regions of mesenchyme. Maisonpierre, et al., *Oncogene* 8: 1631-1637 (1993).

The predominant expression of the TIE receptor in vascular endothelia suggests that TIE plays a role in the development and maintenance of the vascular system. This could include roles in endothelial cell determination, proliferation, differentiation and cell migration and patterning into vascular elements. Analyses of mouse embryos deficient in TIE-2 illustrate its importance in angiogenesis, particularly for vascular network formation in endothelial cells. Sato, T.N., et al., *Nature* 376:70-74 (1995). In the mature vascular system, the TIEs could function in endothelial cell survival, maintenance and response to pathogenic influences.

The TIE receptors are also expressed in primitive hematopoietic stem cells, B cells and a subset of megakaryocytic cells, thus suggesting the role of ligands which bind these receptors in early hematopoiesis, in the differentiation and/or proliferation of B cells, and in the megakaryocytic differentiation pathway. Iwama, et al. *Biochem. Biophys. Research Communications* 195:301-309 (1993); Hashiyama, et al. *Blood* 87:93-101 (1996), Batard, et al. *Blood* 87:2212-2220 (1996).

SUMMARY OF THE INVENTION

The present invention provides for a composition comprising a

modified TIE-2 ligand substantially free of other proteins. As used herein, modified TIE-2 ligand refers to a ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or 5 substitution of one or more amino acids, or by way of tagging, with for example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. Modified TIE-2 ligand also includes a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from 10 the first. By way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For example, other combinations for creating a chimeric TIE-2 ligand are possible, including but not limited to those combinations wherein the 15 first ligand is selected from the group consisting of TL1, TL2, TL3 and TL4, and the second ligand, different from the first ligand, is selected from the group consisting of TL1, TL2, TL3 and TL4.

The invention also provides for an isolated nucleic acid molecule 20 encoding a modified TIE-2 ligand. In one embodiment, the isolated nucleic acid molecule encodes a TIE-2 ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for 25 example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. In another embodiment, the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2

ligand and a portion of a second TIE-2 ligand which is different from the first. By way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For 5 example, other combinations are possible, including but not limited to those combinations wherein the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising a portion of a first ligand selected from the group consisting of TL1, TL2, TL3 and TL4, and a portion of a second ligand, different from the 10 first ligand, selected from the group consisting of TL1, TL2, TL3 and TL4.

The isolated nucleic acid may be DNA, cDNA or RNA. The invention also provides for a vector comprising an isolated nucleic acid molecule 15 encoding a modified TIE-2 ligand. The invention further provides for a host-vector system for the production in a suitable host cell of a polypeptide having the biological activity of a modified TIE-2 ligand. The suitable host cell may be bacterial, yeast, insect or mammalian. The invention also provides for a method of producing a polypeptide 20 having the biological activity of a modified TIE-2 ligand which comprises growing cells of the host-vector system under conditions permitting production of the polypeptide and recovering the polypeptide so produced.

The invention herein described of an isolated nucleic acid 25 molecule encoding a modified TIE-2 ligand further provides for the development of the ligand as a therapeutic for the treatment of patients suffering from disorders involving cells, tissues or organs which express the TIE-2 receptor. The present invention also provides

for an antibody which specifically binds such a therapeutic molecule. The antibody may be monoclonal or polyclonal. The invention also provides for a method of using such a monoclonal or polyclonal antibody to measure the amount of the therapeutic molecule in a 5 sample taken from a patient for purposes of monitoring the course of therapy.

The present invention also provides for an antibody which specifically binds a modified TIE-2 ligand as described herein. The antibody may be monoclonal or polyclonal. Thus the invention further 10 provides for therapeutic compositions comprising an antibody which specifically binds a modified TIE-2 ligand, in a pharmaceutically acceptable vehicle. The invention also provides for a method of blocking blood vessel growth in a mammal by administering an effective amount of a therapeutic composition comprising an antibody 15 which specifically binds a receptor activating modified TIE-2 ligand as described herein, in a pharmaceutically acceptable vehicle.

The invention further provides for therapeutic compositions comprising a modified TIE-2 ligand as described herein, in a pharmaceutically acceptable vehicle. The invention also provides for a 20 method of promoting neovascularization in a patient by administering an effective amount of a therapeutic composition comprising a receptor activating modified TIE-2 ligand as described herein, in a pharmaceutically acceptable vehicle. In one embodiment, the method may be used to promote wound healing. In another embodiment, the 25 method may be used to treat ischemia. In yet another embodiment, a receptor activating modified TIE-2 ligand as described herein is used, alone or in combination with other hematopoietic factors, to promote the proliferation or differentiation of hematopoietic stem cells, B

cells or megakaryocytic cells.

Alternatively, the invention provides that a modified TIE-2 ligand may be conjugated to a cytotoxic agent and a therapeutic composition prepared therefrom. The invention further provides for a receptorbody which specifically binds a modified TIE-2 ligand. The invention further provides for therapeutic compositions comprising a receptorbody which specifically binds a modified TIE-2 ligand in a pharmaceutically acceptable vehicle. The invention also provides for a method of blocking blood vessel growth in a mammal by administering an effective amount of a therapeutic composition comprising a receptorbody which specifically binds a modified TIE-2 ligand in a pharmaceutically acceptable vehicle.

The invention also provides for a TIE-2 receptor antagonist as well as a method of inhibiting TIE-2 biological activity in a mammal comprising administering to the mammal an effective amount of a TIE-2 antagonist. According to the invention, the antagonist may be a modified TIE-2 ligand as described herein which binds to, but does not activate, the TIE-2 receptor.

20

BRIEF DESCRIPTION OF THE FIGURES

FIGURES 1A and 1B - TIE-2 receptorbody (TIE-2 RB) inhibits the development of blood vessels in the embryonic chicken chorioallantoic membrane (CAM). A single piece of resorbable gelatin foam (Gelfoam) soaked with 6 μ g of RB was inserted immediately under the CAM of 1-day chick embryos. After 3 further days of incubation, 4 day old embryos and surrounding CAM were removed and examined. FIGURE 1A:

embryos treated with EHK-1 RB (rEHK-1 ecto/hIgG1 Fc) were viable and possessed normally developed blood vessels in their surrounding CAM. FIGURE 1B : all embryos treated with TIE-2 RB (r TIE-2 ecto / h IgG1 Fc) were dead, diminished in size and were almost completely devoid of surrounding blood vessels.

5 FIGURE 2 - Vector pJFE14.

10 FIGURE 3 - Restriction map of λgt10.

FIGURE 4 - Nucleic acid and deduced amino acid (single letter code) sequences of human TIE-2 ligand 1 from clone λgt10 encoding htie-2 ligand 1.

15 FIGURE 5 - Nucleic acid and deduced amino acid (single letter code) sequences of human TIE-2 ligand 1 from T98G clone.

20 FIGURE 6 - Nucleic acid and deduced amino acid (single letter code) sequences of human TIE-2 ligand 2 from clone pBluescript KS encoding human TIE 2 ligand 2.

FIGURE 7 - Western blot showing activation of TIE-2 receptor by TIE-2 ligand 1 (Lane L1) but not by TIE-2 ligand 2 (Lane L2) or control (Mock).

25 FIGURE 8 - Western blot showing that prior treatment of HAEC cells with excess TIE-2 ligand 2 (Lane 2) antagonizes the subsequent ability of dilute TIE-2 ligand 1 to activate the TIE-2 receptor (TIE2-R) as compared with prior treatment of HAEC cells with MOCK medium (Lane

1).

FIGURE 9 - Western blot demonstrating the ability of TL2 to competitively inhibit TL1 activation of the TIE-2 receptor using the 5 human cell hybrid line, EA.hy926.

FIGURE 10 - Histogram representation of binding to rat TIE-2 IgG immobilized surface by TIE-2 ligand in C2C12 ras, Rat2 ras, SHEP, and T98G concentrated (10x) conditioned medium. Rat TIE-2 (rTIE2) 10 specific binding is demonstrated by the significant reduction in the binding activity in the presence of 25 μ g/ml soluble rat TIE-2 RB as compared to a minor reduction in the presence of soluble trkB RB.

FIGURE 11 - Binding of recombinant human TIE-2 ligand 1 (hTL1) and 15 human TIE-2 ligand 2 (hTL2), in COS cell supernatants, to a human TIE-2 receptorbody (RB) immobilized surface. Human TIE-2-specific binding was determined by incubating the samples with 25 μ g/ml of either soluble human TIE-2 RB or trkB RB; significant reduction in the binding activity is observed only for the samples incubated with human 20 TIE-2 RB.

FIGURE 12 - Western blot showing that TIE-2 receptorbody (denoted TIE-2 RB or, as here, TIE2-Fc) blocks the activation of TIE-2 receptors by TIE-2 ligand 1 (TL1) in HUVEC cells, whereas an unrelated 25 receptorbody (TRKB-Fc) does not block this activation.

FIGURE 13 - Agarose gels showing serial dilutions [undiluted (1) to 10⁻⁴] of the TL1 and TL2 RT-PCR products obtained from E14.5 mouse

fetal liver (Lanes 1- total, Lanes 3- stromal enriched, and Lanes 4- c-kit⁺TER119 hematopoietic precursor cells) and E14.5 mouse fetal thymus (Lanes 2- total).

5 FIGURE 14 - Agarose gels showing serial dilutions [undiluted (1) to 10^{-3}] of the TL1 and TL2 RT-PCR products obtained from E17.5 mouse fetal thymus cortical stromal cells (Lanes 1- CDR1+/A2B5-) and medullary stromal cells (Lane CDR1-/A2B5+).

10 FIGURE 15 - A schematic representation of the hypothesized role of the TIE-2/TIE ligands in angiogenesis. TL1 is represented by (•), TL2 is represented by (*), TIE-2 is represented by (T), VEGF is represented by ([]), and flk-1 (a VEGF receptor) is represented by (Y).

15 FIGURE 16 - *In situ* hybridization slides showing the temporal expression pattern of TIE-2, TL1, TL2, and VEGF during angiogenesis associated with follicular development and corpus luteum formation in the ovary of a rat that was treated with pregnant mare serum. Column 1: Early pre-ovulatory follicle; Column 2: pre-ovulatory follicle; 20 Column 3: early corpus luteum; and Column 4: atretic follicle; Row A: bright field; Row B: VEGF; Row C: TL2; Row D: TL1 and Row E: TIE-2 receptor.

FIGURE 17 - Comparison of amino acid sequences of mature TL1 protein and mature TL2 protein. The TL1 sequence is the same as that set forth in Figure 4, except that the putative leader sequence has been removed. Similarly, the TL2 sequence is the same as that set forth in Figure 6, except that the putative leader sequence has been removed.

Arrows indicate residues Arg49, Cys245 and Arg264 of TL1, which correspond to the residues at amino acid positions 69, 265 and 284, respectively, of TL1 as set forth in Figure 4.

5 FIGURE 18 - Western blot of the covalent multimeric structure of TL1 and TL2 (Panel A) and the interconversion of TL1 and TL2 by the mutation of one cysteine (Panel B).

10 FIGURE 19 - A typical curve of TIE-2-IgG binding to immobilized TL1 in a quantitative cell-free binding assay.

15 FIGURE 20 - A typical curve showing TIE-2 ligand 1 ligandbody comprising the fibrinogen-like domain of the ligand bound to the Fc domain of IgG (TL1-fFc) binding to immobilized TIE-2 ectodomain in a quantitative cell-free binding assay.

20 FIGURE 21 - Nucleotide and deduced amino acid (single letter code) sequences of TIE ligand-3. The coding sequence starts at position 47. The fibrinogen-like domain starts at position 929.

FIGURE 22 - Comparison of Amino Acid Sequences of TIE Ligand Family Members. mTL3 = mouse TIE ligand-3; hTL1 = human TIE-2 ligand1; chTL1 = chicken TIE-2 ligand1; mTL1 = mouse TIE-2 ligand 1; mTL2 = mouse TIE-2 ligand 2; hTL2 = human TIE-2 ligand 2. The boxed regions indicate conserved regions of homology among the family members.

FIGURE 23 - Nucleotide and deduced amino acid (single letter code) sequences of TIE ligand-4. Arrow indicates nucleotide position 569.

FIGURE 24 - Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 1N1C2F (chimera 1). The putative leader sequence is encoded by nucleotides 1-60.

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FIGURE 25 - Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 2N2C1F (chimera 2). The putative leader sequence is encoded by nucleotides 1-48.

10 FIGURE 26 - Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 1N2C2F (chimera 3). The putative leader sequence is encoded by nucleotides 1-60.

15 FIGURE 27 - Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 2N1C1F (chimera 4). The putative leader sequence is encoded by nucleotides 1-48.

DETAILED DESCRIPTION OF THE INVENTION

20

As described in greater detail below, applicants have created novel modified TIE-2 ligands that bind the TIE-2 receptor. The present invention provides for a composition comprising a modified TIE-2 ligand substantially free of other proteins. As used herein, modified

25 TIE-2 ligand refers to a ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for example, the

Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. Modified TIE-2 ligand also includes a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first. By 5 way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For example, other combinations for creating a chimeric TIE-2 ligand are possible, including but not limited to those combinations wherein the 10 first ligand is selected from the group consisting of TL1, TL2, TL3 and TL4, and the second ligand, different from the first ligand, is selected from the group consisting of TL1, TL2, TL3 and TL4.

The invention also provides for an isolated nucleic acid molecule 15 encoding a modified TIE-2 ligand. In one embodiment, the isolated nucleic acid molecule encodes a TIE-2 ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for 20 example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. In another embodiment, the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from 25 the first. By way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For example, other combinations are possible, including but not limited to

those combinations wherein the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising a portion of a first ligand selected from the group consisting of TL1, TL2, TL3 and TL4, and a portion of a second ligand, different from the 5 first ligand, selected from the group consisting of TL1, TL2, TL3 and TL4.

The present invention comprises the modified TIE-2 ligands and their amino acid sequences, as well as functionally equivalent variants 10 thereof, as well as proteins or peptides comprising substitutions, deletions or insertional mutants of the described sequences, which bind TIE-2 receptor and act as agonists or antagonists thereof. Such variants include those in which amino acid residues are substituted for residues within the sequence resulting in a silent change. For 15 example, one or more amino acid residues within the sequence can be substituted by another amino acid(s) of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, 20 the class of nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and 25 histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Also included within the scope of the invention are proteins or

fragments or derivatives thereof which exhibit the same or similar biological activity as the modified TIE-2 ligands described herein, and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Functionally equivalent molecules also include molecules that contain modifications, including N-terminal modifications, which result from expression in a particular recombinant host, such as, for example, N-terminal methylation which occurs in certain bacterial (e.g. E. coli) expression systems.

The present invention also encompasses the nucleotide sequences that encode the proteins described herein as modified TIE-2 ligands, as well as host cells, including yeast, bacteria, viruses, and mammalian cells, which are genetically engineered to produce the proteins, by e.g. transfection, transduction, infection, electroporation, or microinjection of nucleic acid encoding the modified TIE-2 ligands described herein in a suitable expression vector. The present invention also encompasses introduction of the nucleic acid encoding modified TIE-2 ligands through gene therapy techniques such as is described, for example, in Finkel and Epstein FASEB J. 9:843-851 (1995); Guzman, et al. PNAS (USA) 91:10732-10736 (1994).

One skilled in the art will also recognize that the present invention encompasses DNA and RNA sequences that hybridize to a modified TIE-2 ligand encoding nucleotide sequence, under conditions of moderate stringency, as defined in, for example, Sambrook, et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989). Thus, a nucleic acid molecule

contemplated by the invention includes one having a nucleotide sequence deduced from an amino acid sequence of a modified TIE-2 ligand prepared as described herein, as well as a molecule having a sequence of nucleotides that hybridizes to such a nucleotide sequence, 5 and also a nucleotide sequence which is degenerate of the above sequences as a result of the genetic code, but which encodes a ligand that binds TIE-2 receptor and which has an amino acid sequence and other primary, secondary and tertiary characteristics that are sufficiently duplicative of a modified TIE-2 ligand described herein so 10 as to confer on the molecule the same biological activity as the modified TIE-2 ligand described herein.

The present invention provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2. The invention 15 also provides for such a nucleic acid molecule, with a further modification such that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2. 20

25 The present invention also provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-

terminal domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2 and which is further modified to encode a different amino acid instead of the cysteine residue encoded by nucleotides 784-787 as set forth in Figure 27. A serine residue is preferably substituted for the cysteine residue. In another embodiment, the nucleic acid molecule is further modified to encode a different amino acid instead of the arginine residue encoded by nucleotides 199-201 as set forth in Figure 27. A serine residue is preferably substituted for the arginine residue.

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The present invention also provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 which is modified to encode a different amino acid instead of the cysteine residue at amino acid position 245. A serine residue is preferably substituted for the cysteine residue.

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The invention further provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is deleted. The invention also provides for such a nucleic acid molecule further modified so that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is deleted and the portion encoding the fibrinogen-like domain is fused in-frame to a nucleotide sequence encoding a human immunoglobulin gamma-1 constant region (IgG1 Fc).

The invention further provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 2 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2 is deleted. The invention also provides for such a nucleic acid molecule further modified so that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2 is deleted and the portion encoding the fibrinogen-like domain is fused in-frame to a nucleotide sequence encoding a 10 human immunoglobulin gamma-1 constant region (IgG1 Fc).

The invention further provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 2. The invention also provides for such a nucleic acid molecule further modified so that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2. 15 20

The invention further provides for a modified TIE-2 ligand encoded by any of nucleic acid molecules of the invention.

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The present invention also provides for a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first, wherein the

first and second TIE-2 ligands are selected from the group consisting of TIE-2 Ligand-1, TIE-2 Ligand-2, TIE Ligand-3 and TIE Ligand-4.

Preferably, the chimeric TIE ligand comprises at least a portion of TIE-2 Ligand-1 and a portion of TIE-2 Ligand-2.

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The invention also provides a nucleic acid molecule that encodes a chimeric TIE ligand as set forth in Figure 24, 25, 26, or 27. The invention also provides a chimeric TIE ligand as set forth in Figure 24, 25, 26, or 27. The invention further provides a chimeric TIE ligand as 10 set forth in Figure 27, modified to have a different amino acid instead of the cysteine residue encoded by nucleotides 784-787.

Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression 15 vectors encoding a modified TIE-2 ligand using appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination). Expression of a nucleic acid sequence encoding a 20 modified TIE-2 ligand or peptide fragments thereof may be regulated by a second nucleic acid sequence which is operably linked to the a modified TIE-2 ligand encoding sequence such that the modified TIE-2 ligand protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a modified TIE- 25 2 ligand described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the ligand include, but are not limited to the long terminal repeat as described in Squinto et al., (Cell 65:1-20 (1991));

the SV40 early promoter region (Benoist and Chambon, *Nature* 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat, the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., *Cell* 22:787-797 (1980)), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:144-1445 (1981)), the adenovirus promoter, the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39-42 (1982)); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff, et al., *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731 (1978)), or the tac promoter (DeBoer, et al., *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25 (1983)), see also "Useful proteins from recombinant bacteria" in *Scientific American*, 242:74-94 (1980); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals; elastase I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell* 38:639-646 (1984); Ornitz et al., *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, *Hepatology* 7:425-515 (1987); insulin gene control region which is active in pancreatic beta cells [Hanahan, *Nature* 315:115-122 (1985)]; immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:121-132).

1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al, 1987, Genes and Devel. 5:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocytes in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378). The invention further encompasses the production of antisense compounds which are capable of specifically hybridizing with a sequence of RNA encoding a modified TIE-2 ligand to modulate its expression. Ecker, U.S. Patent No. 5,166,195, issued November 24, 1992.

Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising a nucleic acid encoding a modified TIE-2 ligand as described herein, are used to transfect a host and thereby direct expression of such nucleic acid to produce a modified TIE-2 ligand, which may then be recovered in a biologically active form. As used herein, a biologically active form includes a form capable of binding to TIE receptor and causing a biological response such as a differentiated function or influencing the phenotype of the cell expressing the receptor. Such biologically active forms could, for example, induce phosphorylation of the tyrosine kinase domain of TIE receptor. Alternatively, the biological activity may be an effect as an antagonist to the TIE receptor. In alternative

embodiments, the active form of a modified TIE-2 ligand is one that can recognize TIE receptor and thereby act as a targeting agent for the receptor for use in both diagnostics and therapeutics. In accordance with such embodiments, the active form need not confer upon any TIE expressing cell any change in phenotype.

5 Expression vectors containing the gene inserts can be identified by four general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, (c) expression of inserted sequences and (d) PCR detection. In the first approach, the presence of 10 a foreign gene inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted modified TIE-2 ligand encoding gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" 15 gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if a nucleic acid encoding a modified TIE-2 ligand is inserted within the marker gene sequence of the vector, 20 recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of a modified TIE-2 25 ligand gene product, for example, by binding of the ligand to TIE receptor or a portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or by binding to antibodies produced against the modified TIE-2 ligand protein or a portion

thereof. Cells of the present invention may transiently or, preferably, constitutively and permanently express a modified TIE-2 ligand as described herein. In the fourth approach, DNA nucleotide primers can be prepared corresponding to a tie specific DNA sequence. These 5 primers could then be used to PCR a tie gene fragment. (PCR Protocols: A Guide To Methods and Applications, Edited by Michael A. Innis et al., Academic Press (1990)).

The recombinant ligand may be purified by any technique which allows for the subsequent formation of a stable, biologically active 10 protein. Preferably, the ligand is secreted into the culture medium from which it is recovered. Alternatively, the ligand may be recovered from cells either as soluble proteins or as inclusion bodies, from which it may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis in accordance with well known methodology. 15 In order to further purify the ligand, affinity chromatography, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

20 In additional embodiments of the invention, as described in greater detail in the Examples, a modified TIE-2 ligand encoding gene may be used to inactivate or "knock out" an endogenous gene by homologous recombination, and thereby create a TIE ligand deficient cell, tissue, or animal. For example, and not by way of limitation, the recombinant 25 TIE ligand-4 encoding gene may be engineered to contain an insertional mutation, for example the neo gene, which would inactivate the native TIE ligand-4 encoding gene. Such a construct, under the control of a suitable promoter, may be introduced into a cell, such as an embryonic

stem cell, by a technique such as transfection, transduction, or injection. Cells containing the construct may then be selected by G418 resistance. Cells which lack an intact TIE ligand-4 encoding gene may then be identified, e.g. by Southern blotting, PCR detection, Northern blotting or assay of expression. Cells lacking an intact TIE ligand-4 encoding gene may then be fused to early embryo cells to generate transgenic animals deficient in such ligand. Such an animal may be used to define specific in vivo processes, normally dependent upon the ligand.

The present invention also provides for antibodies to a modified TIE-2 ligand described herein which are useful for detection of the ligand in, for example, diagnostic applications. For preparation of monoclonal antibodies directed toward a modified TIE-2 ligand, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, *Nature* 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

The monoclonal antibodies may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:7308-7312; Kozbor et al., 1983, *Immunology Today* 4:72-79; Olsson et al., 1982, *Meth. Enzymol.* 92:3-16). Chimeric antibody

molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

Various procedures known in the art may be used for the 5 production of polyclonal antibodies to epitopes of a modified TIE-2 ligand described herein. For the production of antibody, various host animals, including but not limited to rabbits, mice and rats can be immunized by injection with a modified TIE-2 ligand, or a fragment or derivative thereof. Various adjuvants may be used to increase the 10 immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful 15 human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

A molecular clone of an antibody to a selected a modified TIE-2 ligand epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A 20 Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.

The present invention provides for antibody molecules as well as 25 fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of

the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified 5 by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination ,thereof.

The present invention further encompasses an immunoassay for measuring the amount of a modified TIE-2 ligand in a biological sample 10 by

- a) contacting the biological sample with at least one antibody which specifically binds a modified TIE-2 ligand so that the antibody forms a complex with any modified TIE-2 ligand present in the sample; and
- 15 b) measuring the amount of the complex and thereby measuring the amount of the modified TIE-2 ligand in the biological sample.

The invention further encompasses an assay for measuring the amount of TIE receptor in a biological sample by

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- a) contacting the biological sample with at least one ligand of the invention so that the ligand forms a complex with the TIE receptor; and
- b) measuring the amount of the complex and thereby measuring the amount of the TIE receptor in the biological sample.

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The present invention also provides for the utilization of a modified TIE-2 ligand which activates the TIE-2 receptor as described herein, to support the survival and/or growth and/or migration and/or

differentiation of TIE-2 receptor expressing cells. Thus, the ligand may be used as a supplement to support, for example, endothelial cells in culture.

Further, the creation by applicants of a modified TIE-2 ligand for the TIE-2 receptor enables the utilization of assay systems useful for the identification of agonists or antagonists of the TIE-2 receptor. Such assay systems would be useful in identifying molecules capable of promoting or inhibiting angiogenesis. For example, in one embodiment, antagonists of the TIE-2 receptor may be identified as test molecules that are capable of interfering with the interaction of the TIE-2 receptor with a modified TIE-2 ligand that binds the TIE-2 receptor. Such antagonists are identified by their ability to 1) block the binding of a biologically active modified TIE-2 ligand to the receptor as measured, for example, using BIACore biosensor technology (BIACore; Pharmacia Biosensor, Piscataway, NJ); or 2) block the ability of a biologically active modified TIE-2 ligand to cause a biological response. Such biological responses include, but are not limited to, phosphorylation of the TIE receptor or downstream components of the TIE signal transduction pathway, or survival, growth or differentiation of TIE receptor bearing cells.

In one embodiment, cells engineered to express the TIE receptor may be dependent for growth on the addition of a modified TIE-2 ligand. Such cells provide useful assay systems for identifying additional agonists of the TIE receptor, or antagonists capable of interfering with the activity of the modified TIE-2 ligand on such cells. Alternatively, autocrine cells, engineered to be capable of co-expressing both a modified TIE-2 ligand and receptor, may provide useful systems for assaying potential agonists or antagonists.

Therefore, the present invention provides for introduction of a TIE-2 receptor into cells that do not normally express this receptor, thus allowing these cells to exhibit profound and easily distinguishable responses to a ligand which binds this receptor. The 5 type of response elicited depends on the cell utilized, and not the specific receptor introduced into the cell. Appropriate cell lines can be chosen to yield a response of the greatest utility for assaying, as well as discovering, molecules that can act on tyrosine kinase receptors. The molecules may be any type of molecule, including but 10 not limited to peptide and non-peptide molecules, that will act in systems to be described in a receptor specific manner.

One of the more useful systems to be exploited involves the introduction of a TIE receptor (or a chimeric receptor comprising the extracellular domain of another receptor tyrosine kinase such as, for 15 example, *trkC* and the intracellular domain of a TIE receptor) into a fibroblast cell line (*e.g.*, NIH3T3 cells) thus such a receptor which does not normally mediate proliferative or other responses can, following introduction into fibroblasts, nonetheless be assayed by a variety of well established methods to quantitate effects of fibroblast growth 20 factors (*e.g.* thymidine incorporation or other types of proliferation assays; see van Zoelen, 1990, "The Use of Biological Assays For Detection Of Polypeptide Growth Factors" in *Progress Factor Research*, Vol. 2, pp. 131-152; Zhan and M. Goldfarb, 1986, *Mol. Cell. Biol.*, Vol. 6, pp. 3541-3544). These assays have the added advantage that any 25 preparation can be assayed both on the cell line having the introduced receptor as well as the parental cell line lacking the receptor; only specific effects on the cell line with the receptor would be judged as being mediated through the introduced receptor. Such cells may be

further engineered to express a modified TIE-2 ligand, thus creating an autocrine system useful for assaying for molecules that act as antagonists/agonists of this interaction. Thus, the present invention provides for host cells comprising nucleic acid encoding a modified 5 TIE-2 ligand and nucleic acid encoding TIE receptor.

The TIE receptor/modified TIE-2 ligand interaction also provides a useful system for identifying small molecule agonists or antagonists of the TIE receptor. For example, fragments, mutants or derivatives of a modified TIE-2 ligand may be identified that bind TIE receptor but do 10 not induce any other biological activity. Alternatively, the characterization of a modified TIE-2 ligand enables the further characterization of active portions of the molecule. Further, the identification of a ligand enables the determination of the X-ray crystal structure of the receptor/ligand complex, thus enabling 15 identification of the binding site on the receptor. Knowledge of the binding site will provide useful insight into the rational design of novel agonists and antagonists.

The specific binding of a test molecule to TIE receptor may be measured in a number of ways. For example, the actual binding of test 20 molecule to cells expressing TIE may be detected or measured, by detecting or measuring (i) test molecule bound to the surface of intact cells; (ii) test molecule cross-linked to TIE protein in cell lysates; or (iii) test molecule bound to TIE in vitro. The specific interaction between test molecule and TIE may be evaluated by using reagents that 25 demonstrate the unique properties of that interaction.

As a specific, nonlimiting example, the methods of the invention may be used as follows. Consider a case in which a modified TIE-2 ligand in a sample is to be measured. Varying dilutions of the sample

(the test molecule), in parallel with a negative control (NC) containing no modified TIE-2 ligand activity, and a positive control (PC) containing a known amount of a modified TIE-2 ligand, may be exposed to cells that express TIE in the presence of a detectably labeled 5 modified TIE-2 ligand (in this example, radioiodinated ligand). The amount of modified TIE-2 ligand in the test sample may be evaluated by determining the amount of ^{125}I -labeled modified TIE-2 ligand that binds to the controls and in each of the dilutions, and then comparing the sample values to a standard curve. The more modified TIE-2 ligand 10 in the sample, the less ^{125}I -ligand that will bind to TIE.

The amount of ^{125}I -ligand bound may be determined by measuring the amount of radioactivity per cell, or by cross-linking a modified TIE-2 ligand to cell surface proteins using DSS, as described in Meakin and Shooter, 1991, *Neuron* 6:153-163, and detecting the amount of 15 labeled protein in cell extracts using, for example, SDS polyacrylamide gel electrophoresis, which may reveal a labeled protein having a size corresponding to TIE receptor/modified TIE-2 ligand. The specific test molecule/TIE interaction may further be tested by adding to the assays various dilutions of an unlabeled control ligand that does not bind the 20 TIE receptor and therefore should have no substantial effect on the competition between labeled modified TIE-2 ligand and test molecule for TIE binding. Alternatively, a molecule known to be able to disrupt TIE receptor/modified TIE-2 ligand binding, such as, but not limited to, anti-TIE antibody, or TIE receptorbody as described herein, may be 25 expected to interfere with the competition between ^{125}I -modified TIE-2 ligand and test molecule for TIE receptor binding.

Detectably labeled modified TIE-2 ligand includes, but is not limited to, a modified TIE-2 ligand linked covalently or noncovalently

to a radioactive substance, a fluorescent substance, a substance that has enzymatic activity, a substance that may serve as a substrate for an enzyme (enzymes and substrates associated with colorimetrically detectable reactions are preferred) or to a substance that can be 5 recognized by an antibody molecule that is preferably a detectably labeled antibody molecule.

Alternatively, the specific binding of test molecule to TIE may be measured by evaluating the secondary biological effects of a modified TIE-2 ligand/TIE receptor binding, including, but not limited 10 to, cell growth and/or differentiation or immediate early gene expression or phosphorylation of TIE. For example, the ability of the test molecule to induce differentiation can be tested in cells that lack tie and in comparable cells that express tie; differentiation in tie-expressing cells but not in comparable cells that lack tie would be 15 indicative of a specific test molecule/TIE interaction. A similar analysis could be performed by detecting immediate early gene (e.g. fos and jun) induction in tie-minus and tie-plus cells, or by detecting phosphorylation of TIE using standard phosphorylation assays known in the art. Such analysis might be useful in identifying agonists or 20 antagonists that do not competitively bind to TIE.

Similarly, the present invention provides for a method of identifying a molecule that has the biological activity of a modified TIE-2 ligand comprising (i) exposing a cell that expresses tie to a test molecule and (ii) detecting the specific binding of the test molecule to 25 TIE receptor, in which specific binding to TIE positively correlates with TIE-like activity. Specific binding may be detected by either assaying for direct binding or the secondary biological effects of binding, as discussed supra. Such a method may be particularly useful

in identifying new members of the TIE ligand family or, in the pharmaceutical industry, in screening a large array of peptide and non-peptide molecules (e.g., peptidomimetics) for TIE associated biological activity. In a preferred, specific, nonlimiting embodiment of the invention, a large grid of culture wells may be prepared that contain, in alternate rows, PC12 (or fibroblasts, see infra) cells that are either tie-minus or engineered to be tie-plus. A variety of test molecules may then be added such that each column of the grid, or a portion thereof, contains a different test molecule. Each well could then be scored for the presence or absence of growth and/or differentiation. An extremely large number of test molecules could be screened for such activity in this manner.

In additional embodiments, the invention provides for methods of detecting or measuring TIE ligand-like activity or identifying a molecule as having such activity comprising (i) exposing a test molecule to a TIE receptor protein in vitro under conditions that permit binding to occur and (ii) detecting binding of the test molecule to the TIE receptor protein, in which binding of test molecule to TIE receptor correlates with TIE ligand-like activity. According to such methods, the TIE receptor may or may not be substantially purified, may be affixed to a solid support (e.g. as an affinity column or as an ELISA assay), or may be incorporated into an artificial membrane. Binding of test molecule to TIE receptor may be evaluated by any method known in the art. In preferred embodiments, the binding of test molecule may be detected or measured by evaluating its ability to compete with detectably labeled known TIE ligands for TIE receptor binding.

The present invention also provides for a method of detecting the

ability of a test molecule to function as an antagonist of TIE ligand-like activity comprising detecting the ability of the molecule to inhibit an effect of TIE ligand binding to TIE receptor on a cell that expresses the receptor. Such an antagonist may or may not interfere with TIE receptor/modified TIE-2 ligand binding. Effects of a modified TIE-2 ligand binding to TIE receptor are preferably biological or biochemical effects, including, but not limited to, cell survival or proliferation, cell transformation, immediate early gene induction, or TIE phosphorylation.

10 The invention further provides for both a method of identifying antibodies or other molecules capable of neutralizing the ligand or blocking binding to the receptor, as well as the molecules identified by the method. By way of nonlimiting example, the method may be performed via an assay which is conceptually similar to an ELISA assay. For example, TIE receptorbody may be bound to a solid support, such as a plastic multiwell plate. As a control, a known amount of a modified TIE-2 ligand which has been Myc-tagged may then be introduced to the well and any tagged modified TIE-2 ligand which binds the receptorbody may then be identified by means of a reporter 15 antibody directed against the Myc-tag. This assay system may then be used to screen test samples for molecules which are capable of i) binding to the tagged ligand or ii) binding to the receptorbody and thereby blocking binding to the receptorbody by the tagged ligand. For example, a test sample containing a putative molecule of interest 20 25 together with a known amount of tagged ligand may be introduced to the well and the amount of tagged ligand which binds to the receptorbody may be measured. By comparing the amount of bound tagged ligand in the test sample to the amount in the control, samples

containing molecules which are capable of blocking ligand binding to the receptor may be identified. The molecules of interest thus identified may be isolated using methods well known to one of skill in the art.

Once a blocker of ligand binding is found, one of skill in the art would know to perform secondary assays to determine whether the blocker is binding to the receptor or to the ligand, as well as assays to determine if the blocker molecule can neutralize the biological activity of the ligand. For example, by using a binding assay which employs BIACore biosensor technology (or the equivalent), in which either TIE receptorbody or a modified TIE-2 ligand or ligandbody is covalently attached to a solid support (e.g. carboxymethyl dextran on a gold surface), one of skill in the art would be able to determine if the blocker molecule is binding specifically to the ligand, ligandbody or to the receptorbody. To determine if the blocker molecule can neutralize the biological activity of the ligand, one of skill in the art could perform a phosphorylation assay (see Example 5) or alternatively, a functional bioassay, such as a survival assay, by using primary cultures of, for example, endothelial cells. Alternatively, a blocker molecule which binds to the receptorbody could be an agonist and one of skill in the art would know to how to determine this by performing an appropriate assay for identifying additional agonists of the TIE receptor.

In addition, the invention further contemplates compositions wherein the TIE ligand is the receptor binding domain of a TIE-2 ligand described herein. For example, TIE-2 ligand 1 contains a "coiled coil" domain (beginning at the 5' end and extending to the nucleotide at about position 1160 of Figure 4 and about position 1157 of Figure 5)

and a fibrinogen-like domain (which is encoded by the nucleotide sequence of Figure 4 beginning at about position 1161 and about position 1158 of Figure 5). The fibrinogen-like domain of TIE-2 ligand 2 is believed to begin on or around the same amino acid sequence as in ligand 1 (FRDCA) which is encoded by nucleotides beginning around 5 1197 of Figure 6. The fibrinogen-like domain of TIE ligand-3 is believed to begin on or around the amino acid sequence which is encoded by nucleotides beginning around position 929 as set forth in Figure 21. Multimerization of the coiled coil domains during 10 production of the ligand hampers purification. As described in Example 19, Applicants have discovered, however, that the fibrinogen-like domain comprises the TIE-2 receptor binding domain. The monomeric forms of the fibrinogen-like domain do not, however, appear to bind the receptor. Studies utilizing myc-tagged fibrinogen-like domain, 15 which has been "clustered" using anti-myc antibodies, do bind the TIE-2 receptor. [Methods of production of "clustered ligands and ligandbodies are described in Davis, et al. Science 266:816-819 (1994)]. Based on these finding, applicants produced "ligandbodies" which comprise the fibrinogen-like domain of the TIE-2 ligands 20 coupled to the Fc domain of IgG ("fFc's"). These ligandbodies, which form dimers, efficiently bind the TIE-2 receptor. Accordingly, the present invention contemplates the production of modified TIE ligandbodies which may be used as targeting agents, in diagnostics or in therapeutic applications, such as targeting agents for tumors and/or 25 associated vasculature wherein a TIE antagonist is indicated.

The invention herein further provides for the development of the ligand, a fragment or derivative thereof, or another molecule which is a receptor agonist or antagonist, as a therapeutic for the treatment of

patients suffering from disorders involving cells, tissues or organs which express the TIE receptor. Such molecules may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

5 Because TIE receptor has been identified in association with endothelial cells and, as demonstrated herein, blocking of TIE-2 ligand 1 appears to prevent vascularization, applicants expect that a modified TIE-2 ligand described herein may be useful for the induction 10 of vascularization in diseases or disorders where such vascularization is indicated. Such diseases or disorders would include wound healing, ischaemia and diabetes. The ligands may be tested in animal models and used therapeutically as described for other agents, such as vascular endothelial growth factor (VEGF), another endothelial cell-specific factor that is angiogenic. Ferrara, et al. U.S. Patent No. 15 5,332,671 issued July 26, 1994. The Ferrara reference, as well as other studies, describe in vitro and in vivo studies that may be used to demonstrate the effect of an angiogenic factor in enhancing blood flow to ischemic myocardium, enhancing wound healing, and in other therapeutic settings wherein neoangiogenesis is desired. [see Sudo, et 20 al. European Patent Application 0 550 296 A2 published July 7, 1993; Banai, et al. Circulation 89:2183-2189 (1994); Unger, et al. Am. J. Physiol. 266:H1588-H1595 (1994); Lazarous, et al. Circulation 91:145-153 (1995)]. According to the invention, a modified TIE-2 ligand may be used alone or in combination with one or more additional 25 pharmaceutically active compounds such as, for example, VEGF or basic fibroblast growth factor (bFGF), as well as cytokines, neurotrophins, etc.

Conversely, antagonists of the TIE receptor, such as modified

TIE-2 ligands which bind but do not activate the receptor as described herein, receptorbodies as described herein in Examples 2 and 3, and TIE-2 ligand 2 as described in Example 9, would be useful to prevent or attenuate vascularization, thus preventing or attenuating, for example, tumor growth. These agents may be used alone or in combination with other compositions, such as anti-VEGF antibodies, that have been shown to be useful in treating conditions in which the therapeutic intent is to block angiogenesis. Applicants expect that a modified TIE-2 ligand described herein may also be used in combination with agents, such as cytokine antagonists such as IL-6 antagonists, that are known to block inflammation.

For example, applicants have determined that TIE ligands are expressed in cells within, or closely associated with, tumors. For example, TIE-2 ligand 2 appears to be tightly associated with tumor endothelial cells. Accordingly, it and other TIE antagonists may also be useful in preventing or attenuating, for example, tumor growth. In addition, TIE ligands or ligandbodies may be useful for the delivery of toxins to a receptor bearing cell. Alternatively, other molecules, such as growth factors, cytokines or nutrients, may be delivered to a TIE receptor bearing cell via TIE ligands or ligandbodies. TIE ligands or ligandbodies such as modified TIE-2 ligand described herein may also be used as diagnostic reagents for TIE receptor, to detect the receptor in vivo or in vitro. Where the TIE receptor is associated with a disease state, TIE ligands or ligandbodies such as a modified TIE-2 ligand may be useful as diagnostic reagents for detecting the disease by, for example, tissue staining or whole body imaging. Such reagents include radioisotopes, flurochromes, dyes, enzymes and biotin. Such diagnostics or targeting agents may be prepared as described in

Alitalo, et al. WO 95/26364 published October 5, 1995 and Burrows, F. and P. Thorpe, PNAS (USA) 90:8996-9000 (1993) which is incorporated herein in its entirety.

In other embodiments, the TIE ligands, a receptor activating modified TIE-2 ligand described herein are used as hematopoietic factors. A variety of hematopoietic factors and their receptors are involved in the proliferation and/or differentiation and/or migration of the various cells types contained within blood. Because the TIE receptors are expressed in early hematopoietic cells, the TIE ligands are expected to play a comparable role in the proliferation or differentiation or migration of these cells. Thus, for example, TIE containing compositions may be prepared, assayed, examined in in vitro and in vivo biological systems and used therapeutically as described in any of the following: Sousa, U.S. Patent No. 4,810,643, Lee, et al., Proc. Natl. Acad. Sci. USA 82:4360-4364 (1985) Wong, et al. Science, 228:810-814 (1985); Yokota, et al. Proc. Natl. Acad. Sci (USA) 81:1070 (1984); Bosselman, et al. WO 9105795 published May 2, 1991 entitled "Stem Cell Factor" and Kirkness, et al. WO 95/19985 published July 27, 1995 entitled "Haemopoietic Maturation Factor".

Accordingly, receptor activating modified TIE-2 ligand may be used to diagnose or treat conditions in which normal hematopoiesis is suppressed, including, but not limited to anemia, thrombocytopenia, leukopenia and granulocytopenia. In a preferred embodiment, receptor activating modified TIE-2 ligand may be used to stimulate differentiation of blood cell precursors in situations where a patient has a disease, such as acquired immune deficiency syndrome (AIDS) which has caused a reduction in normal blood cell levels, or in clinical settings in which enhancement of hematopoietic populations is

desired, such as in conjunction with bone marrow transplant, or in the treatment of aplasia or myelosuppression caused by radiation, chemical treatment or chemotherapy.

The receptor activating modified TIE-2 ligands of the present invention may be used alone, or in combination with another pharmaceutically active agent such as, for example, cytokines, neurotrophins, interleukins, etc. In a preferred embodiment, the ligands may be used in conjunction with any of a number of the above referenced factors which are known to induce stem cell or other hematopoietic precursor proliferation, or factors acting on later cells in the hematopoietic pathway, including, but not limited to, hemopoietic maturation factor, thrombopoietin, stem cell factor, erythropoietin, G-CSF, GM-CSF, etc.

In an alternative embodiment, TIE receptor antagonists are used to diagnose or treat patients in which the desired result is inhibition of a hematopoietic pathway, such as for the treatment of myeloproliferative or other proliferative disorders of blood forming organs such as thrombocythemas, polycythemias and leukemias. In such embodiments, treatment may comprise use of a therapeutically effective amount of the a modified TIE-2 ligand, TIE antibody, TIE receptorbody, a conjugate of a modified TIE-2 ligand, or a ligandbody or fFC as described herein.

The present invention also provides for pharmaceutical compositions comprising a modified TIE-2 ligand or ligandbodies described herein, peptide fragments thereof, or derivatives in a pharmacologically acceptable vehicle. The modified TIE-2 ligand proteins, peptide fragments, or derivatives may be administered systemically or locally. Any appropriate mode of administration

known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

5 The present invention also provides for an antibody which specifically binds such a therapeutic molecule. The antibody may be monoclonal or polyclonal. The invention also provides for a method of using such a monoclonal or polyclonal antibody to measure the amount of the therapeutic molecule in a sample taken from a patient for 10 purposes of monitoring the course of therapy.

The invention further provides for a therapeutic composition comprising a modified TIE-2 ligand or ligandbody and a cytotoxic agent conjugated thereto. In one embodiment, the cytotoxic agent may be a radioisotope or toxin.

15 The invention also provides for an antibody which specifically binds a modified TIE-2 ligand. The antibody may be monoclonal or polyclonal.

The invention further provides for a method of purifying a modified TIE-2 ligand comprising:

20 a) coupling at least one TIE binding substrate to a solid matrix;

b) incubating the substrate of a) with a cell lysate so that the substrate forms a complex with any modified TIE-2 ligand in the cell lysate;

25 c) washing the solid matrix; and

d) eluting the modified TIE-2 ligand from the coupled substrate.

The substrate may be any substance that specifically binds the modified TIE-2 ligand. In one embodiment, the substrate is selected from the group consisting of anti-modified TIE-2 ligand antibody, TIE receptor and TIE receptorbody. The invention further provides for a receptorbody which specifically binds a modified TIE-2 ligand, as well as a therapeutic composition comprising the receptorbody in a pharmaceutically acceptable vehicle, and a method of blocking blood vessel growth in a human comprising administering an effective amount of the therapeutic composition.

10 The invention also provides for a therapeutic composition comprising a receptor activating modified TIE-2 ligand or ligandbody in a pharmaceutically acceptable vehicle, as well as a method of promoting neovascularization in a patient comprising administering to the patient an effective amount of the therapeutic composition.

15 In addition, the present invention provides for a method for identifying a cell which expresses TIE receptor which comprises contacting a cell with a detectably labeled modified TIE-2 ligand or ligandbody, under conditions permitting binding of the detectably labeled ligand to the TIE receptor and determining whether the 20 detectably labeled ligand is bound to the TIE receptor, thereby identifying the cell as one which expresses TIE receptor. The present invention also provides for a therapeutic composition comprising a modified TIE-2 ligand or ligandbody and a cytotoxic agent conjugated thereto. The cytotoxic agent may be a radioisotope or toxin.

25 The invention also provides a method of detecting expression of a modified TIE-2 ligand by a cell which comprises obtaining mRNA from the cell, contacting the mRNA so obtained with a labeled nucleic acid molecule encoding a modified TIE-2 ligand, under hybridizing

conditions, determining the presence of mRNA hybridized to the labeled molecule, and thereby detecting the expression of a modified TIE-2 ligand in the cell.

The invention further provides a method of detecting expression 5 of a modified TIE-2 ligand in tissue sections which comprises contacting the tissue sections with a labeled nucleic acid molecule encoding a modified TIE-2 ligand, under hybridizing conditions, determining the presence of mRNA hybridized to the labelled molecule, and thereby detecting the expression of a modified TIE-2 ligand in 10 tissue sections.

EXAMPLE 1 - IDENTIFICATION OF THE ABAE CELL LINE AS
REPORTER CELLS FOR THE TIE-2 RECEPTOR

Adult BAE cells are registered in the European Cell Culture 15 Repository, under ECACC#92010601. (See PNAS 75:2621 (1978)). Northern (RNA) analyses revealed moderate levels of tie-2 transcripts in the ABAE (Adult Bovine Arterial Endothelial) cell line, consistent with in situ hybridization results that demonstrated almost exclusive localization of tie-2 RNAs to vascular endothelial cells. We therefore 20 examined ABAE cell lysates for the presence of TIE-2 protein, as well as the extent to which this TIE-2 protein is tyrosine-phosphorylated under normal versus serum-deprived growth conditions. ABAE cell lysates were harvested and subjected to immunoprecipitation, followed by Western blot analyses of immunoprecipitated proteins 25 with TIE-2 specific and phosphotyrosine-specific antisera. Omission or inclusion of TIE-2 peptides as specific blocking molecules during TIE-2 immunoprecipitation allowed unambiguous identification of TIE-2 as a moderately detectable protein of ~150 kD whose steady-state

phosphotyrosine levels diminish to near undetectable levels by prior serum-starvation of the cells.

Culture of ABAE cells and harvest of cell lysates was done as follows. Low-passage-number ABAE cells were plated as a monolayer 5 at a density of 2×10^6 cells/150mm plastic petri plate (Falcon) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum (10 % BCS), 2 mM L-glutamine (Q) and 1% each of penicillin and streptomycin (P-S) in an atmosphere of 5% CO_2 . Prior to harvest of cell lysates, cells were serum-starved for 24 hours in 10 DMEM/Q/P-S, followed by aspiration of the medium and rinsing of the plates with ice-cold phosphate buffered saline (PBS) supplemented with sodium orthovanadate, sodium fluoride and sodium benzamidine. Cells were lysed in a small volume of this rinse buffer that had been supplemented with 1% NP40 detergent and the protease inhibitors 15 PMSF and aprotinin. Insoluble debris was removed from the cell lysates by centrifugation at 14,000 xG for 10 minutes, at 4°C and the supernatants were subjected to immunoprecipitation with antisera specific for TIE-2 receptor, with or without the presence of blocking peptides added to ~20 $\mu\text{g}/\text{ml}$ lysate. Immunoprecipitated proteins 20 were resolved by PAGE (7.5% Laemmli gel), and then electro-transferred to PVDF membrane and incubated either with various TIE-2- or phosphotyrosine-specific antisera. TIE-2 protein was visualized by incubation of the membrane with HRP-linked secondary antisera followed by treatment with ECL reagent (Amersham).

25

EXAMPLE 2 - CLONING AND EXPRESSION OF TIE-2 RECEPTORBODY
FOR AFFINITY-BASED STUDY OF TIE-2 LIGAND

INTERACTIONS

An expression construct was created that would yield a secreted protein consisting of the entire extracellular portion of the rat TIE-2 receptor fused to the human immunoglobulin gamma-1 constant region (IgG1 Fc). This fusion protein is called a TIE-2 "receptorbody" (RB), and would be normally expected to exist as a dimer in solution based on formation of disulfide linkages between individual IgG1 Fc tails.

The Fc portion of the TIE-2 RB was prepared as follows. A DNA fragment encoding the Fc portion of human IgG1 that spans from the hinge region to the carboxy-terminus of the protein, was amplified from human placental cDNA by PCR with oligonucleotides corresponding to the published sequence of human IgG1; the resulting DNA fragment was cloned in a plasmid vector. Appropriate DNA restriction fragments from a plasmid encoding the full-length TIE-2 receptor and from the human IgG1 Fc plasmid were ligated on either side of a short PCR-derived fragment that was designed so as to fuse, in-frame, the TIE-2 and human IgG1 Fc protein-coding sequences.

Thus, the resulting TIE-2 ectodomain-Fc fusion protein precisely substituted the IgG1 Fc in place of the region spanning the TIE-2 transmembrane and cytoplasmic domains. An alternative method of preparing RBs is described in Goodwin, et. al. Cell 73:447-456 (1993).

Milligram quantities of TIE-2 RB were obtained by cloning the TIE-2 RB DNA fragment into the pVL1393 baculovirus vector and subsequently infecting the Spodoptera frugiperda SF-21AE insect cell line. Alternatively, the cell line SF-9 (ATCC Accession No. CRL-1711) or the cell line BTI-TN-5b1-4 may be used. DNA encoding the TIE-2 RB was cloned as an Eco RI-NotI fragment into the baculovirus transfer

plasmid pVL1393. Plasmid DNA purified by cesium chloride density gradient centrifugation was recombined into viral DNA by mixing 3 µg of plasmid DNA with 0.5 µg of Baculo-Gold DNA (Pharminigen), followed by introduction into liposomes using 30µg Lipofectin (GIBCO-BRL). DNA-liposome mixtures were added to SF-21AE cells (2x 10⁶ cells/60mm dish) in TMN-FH medium (Modified Grace's Insect Cell Medium (GIBCO-BRL) for 5 hours at 27°C, followed by incubation at 27°C for 5 days in TMN-FH medium supplemented with 5% fetal calf serum. Tissue culture medium was harvested for plaque purification of recombinant viruses, which was carried out using methods previously described (O'Reilly, D.R., L.K. Miller, and V.A. Luckow, Baculovirus Expression Vectors - A Laboratory Manual, 1992, New York: W.H. Freeman) except that the agarose overlay contained 125 µg/mL X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; GIBCO-BRL).

After 5 days of incubation at 27°C, non-recombinant plaques were scored by positive chromogenic reaction to the X-gal substrate, and their positions marked. Recombinant plaques were then visualized by addition of a second overlay containing 100 µg/mL MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide; Sigma).

Putative recombinant virus plaques were picked by plug aspiration, and purified by multiple rounds of plaque isolation to assure homogeneity. Virus stocks were generated by serial, low-multiplicity passage of plaque-purified virus. Low passage stocks of one virus clone (vTIE-2 receptorbody) were produced.

SF-21AE cells were cultured in serum free medium (SF-900 II, Gibco BRL) containing 1X antibiotic/antimycotic solution (Gibco BRL) and 25 mg/L Gentamycin (Gibco BRL). Pluronic F-68 was added as a

surfactant to a final concentration of 1g/L. Cultures (4L) were raised in a bioreactor (Artisan Cell Station System) for at least three days prior to infection. Cells were grown at 27°C, with gassing to 50 % dissolved oxygen, at a gas flow rate of 80 mL/min (aeration at a 5 sparge ring). Agitation was by means of a marine impeller at a rate of 100 rpm. Cells were harvested in mid-logarithmic growth phase (~2 $\times 10^6$ cells/mL), concentrated by centrifugation, and infected with 5 plaque forming units of vTIE-2 receptorbody per cell. Cells and inoculum were brought to 400mL with fresh medium, and virus was 10 adsorbed for 2 hours at 27°C in a spinner flask. The culture was then resuspended in a final volume of 8L with fresh serum-free medium, and the cells incubated in the bioreactor using the previously described conditions.

Culture medium from vTIE-2 receptorbody-infected SF21AE cells 15 were collected by centrifugation (500x g, 10 minutes) at 72 hours post-infection. Cell supernatants were brought to pH 8 with NaOH. EDTA was added to a final concentration of 10 mM and the supernatant pH was readjusted to 8. Supernatants were filtered (0.45 μ m, Millipore) and loaded on a protein A column (protein A sepharose 4 fast 20 flow or HiTrap protein A, both from Pharmacia). The column was washed with PBS containing 0.5 M NaCl until the absorbance at 280 nm decreased to baseline. The column was washed in PBS and eluted with 0.5 M acetic acid. Column fractions were immediately neutralized by eluting into tubes containing 1 M Tris pH 9. The peak fractions 25 containing the TIE-2 receptorbody were pooled and dialyzed versus PBS.

EXAMPLE 3 - DEMONSTRATION THAT TIE-2 HAS A CRITICAL
ROLE IN DEVELOPMENT OF THE VASCULATURE

Insight into the function of TIE-2 was gained by introduction of
5 "excess" soluble TIE-2 receptorbody (TIE-2 RB) into a developing
system. The potential ability of TIE-2 RB to bind, and thereby
neutralize, available TIE-2 ligand could result in an observable
disruption of normal vascular development and characterization of the
ligand. To examine whether TIE-2 RB could be used to disrupt vascular
10 development in early chick embryos, small pieces of a biologically
resorbable foam were soaked with TIE-2 RB and inserted immediately
beneath the chorioallantoic membrane at positions just lateral to the
primitive embryo.

Early chicken embryos develop atop the yolk from a small disk of
15 cells that is covered by the chorioallantoic membrane (CAM). The
endothelial cells that will come to line the vasculature in the embryo
arise from both extra- and intra-embryonic cell sources. Extra-
embryonically-derived endothelial cells, which provide the major
source of endothelial cells in the embryo, originate from accretions of
20 mesenchyme that are situated laterally around the embryo-proper, just
underneath the CAM. As these mesenchyme cells mature, they give rise
to a common progenitor of both the endothelial and hematopoietic cell
lineages, termed the hemangioblast. In turn, the hemangioblast gives
rise to a mixed population of angioblasts (the endothelial cell
25 progenitor) and hematoblasts (the pluripotential hematopoietic
precursor). Formation of rudiments of the circulatory system begins
when endothelial cell progeny segregate to form a one-cell-thick
vesicle that surrounds the primitive blood cells. Proliferation and

migration of these cellular components eventually produces a vast network of blood-filled microvessels under the CAM that will ultimately invade the embryo to join with limited, intra-embryonically-derived vascular elements.

5 Newly fertilized chicken eggs obtained from Spafas, Inc. (Boston, MA) were incubated at 99.5°F, 55 % relative humidity. At about 24 hrs. of development, the egg shell was wiped down with 70% ethanol and a dentist's drill was used to make a 1.5 cm. hole in the blunt apex of each egg. The shell membrane was removed to reveal an air space
10 directly above the embryo. Small rectangular pieces of sterile Gelfoam (Upjohn) were cut with a scalpel and soaked in equal concentrations of either TIE-2- or EHK-1 receptorbody. EHK-1 receptorbody was made as set forth in Example 2 using the EHK-1 extracellular domain instead of the TIE-2 extracellular domain
15 (Maisonpierre et al., Oncogene 8:3277-3288 (1993). Each Gelfoam piece absorbed approximately 6 µg of protein in 30 µl. Sterile watchmakers forceps were used to make a small tear in the CAM at a position several millimeters lateral to the primitive embryo. The majority of the piece of RB-soaked Gelfoam was inserted under the CAM and the
20 egg shell was sealed over with a piece of adhesive tape. Other similarly-staged eggs were treated in parallel with RB of the unrelated, neuronally expressed receptor tyrosine kinase, EHK-1 (Maisonpierre et al., Oncogene 8:3277-3288 (1993). Development was allowed to proceed for 4 days and then the embryos were examined by
25 visual inspection. Embryos were removed by carefully breaking the shells in dishes of warmed PBS and carefully cutting away the embryo with surrounding CAM. Of 12 eggs treated with each RB, 6 TIE-2 RB and 5 EHK-1 RB treated embryos had developed beyond the stage

observed at the start of the experiment. A dramatic difference was seen between these developed embryos, as shown in Figures 1A and 1B. Those treated with EHK-1 RB appeared to have developed relatively normally. Four out of five EHK-1 embryos were viable as judged by the 5 presence of a beating heart. Furthermore, the extra-embryonic vasculature, which is visually obvious due to the presence of red blood cells, was profuse and extended several centimeters laterally under the CAM. By contrast, those treated with TIE-2 RB were severely stunted, ranging from 2-5 mm. in diameter, as compared with more 10 than 10 mm in diameter for the EHK-1 RB embryos. All of the TIE-2 RB treated embryos were dead and their CAMs were devoid of blood vessels. The ability of TIE-2 RB to block vascular development in the chicken demonstrates that TIE-2 ligand is necessary for development of the vasculature.

15

EXAMPLE 4 - IDENTIFICATION OF A TIE-2-SPECIFIC BINDING
ACTIVITY IN CONDITIONED MEDIUM FROM THE ras
ONCOGENE-TRANSFORMED C2C12 MOUSE MYOBLAST
CELL LINE

20

Screening of ten-fold-concentrated cell-conditioned media (10X CCM) from various cell lines for the presence of soluble, TIE-2-specific binding activity (BIAcore; Pharmacia Biosensor, Piscataway, NJ) revealed binding activity in serum-free medium from oncogenic-ras-transformed C2C12 cells (C2C12-ras), RAT 2-ras (which is a ras 25 transformed fibroblast cell line), human glioblastoma T98G and the human neuroblastoma cell line known as SHEP-1.

The C2C12-ras 10X CCM originated from a stably transfected line

of C2C12 myoblasts that was oncogenically transformed by transfection with the T-24 mutant of H-ras by standard calcium phosphate-based methods. An SV40 based neomycin-resistance expression plasmid was physically linked with the ras expression plasmid in order to permit selection of transfected clones. Resulting G418-resistant ras-C2C12 cells were routinely maintained as a monolayer on plastic dishes in DMEM/glutamine/penicillin-streptomycin supplemented with 10 % fetal calf serum (FCS). Serum-free C2C12-ras 10X CCM was made by plating the cells at 60% confluence in a serum free defined media for 12 hours. [Zhan and Goldfarb, Mol. Cell. Biol. 6: 3541-3544 (1986)); Zhan, et al. Oncogene 1: 369-376 (1987)]. The medium was discarded and replaced with fresh DMEM/Q/P-S for 24 hours. This medium was harvested and cells were re-fed fresh DMEM/Q/P-S, which was also harvested after a further 24 hours. These CCM were supplemented with the protease inhibitors PMSF (1mM) and aprotinin (10 μ g/ml), and ten-fold concentrated on sterile size-exclusion membranes (Amicon). TIE-2-binding activity could be neutralized by incubation of the medium with an excess of TIE-2 RB, but not by incubation with EHK-1 RB, prior to BIACore analysis.

Binding activity of the 10x CCM was measured using biosensor technology (BIACore; Pharmacia Biosensor, Piscataway, NJ) which monitors biomolecular interactions in real-time via surface plasmon resonance. Purified TIE-2 RB was covalently coupled through primary amines to the carboxymethyl dextran layer of a CM5 research grade sensor chip (Pharmacia Biosensor; Piscataway, NJ). The sensor chip surface was activated using a mixture of N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide (EDC), followed

by immobilization of TIE-2 RB (25 μ g/mL, pH 4.5) and deactivation of unreacted sites with 1.0 M ethanolamine (pH 8.5). A negative control surface of the EHK-1 receptorbody was prepared in a similar manner.

The running buffer used in the system was HBS (10 mM Hepes, 3.4
5 mM EDTA, 150 mM NaCl, 0.005% P20 surfactant, pH 7.4). The 10x CCM
samples were centrifuged for 15 min at 4° C and further clarified
using a sterile, low protein-binding 0.45 μ m filter (Millipore; Bedford,
MA). Dextran (2mg/ml) and P20 surfactant (0.005%) were added to
each CCM sample. Aliquots of 40 μ L were injected across the
10 immobilized surface (either TIE-2 or EHK-1) at a flow rate of 5 μ L/min
and the receptor binding was monitored for 8 min. The binding activity
(resonance units, RU) was measured as the difference between a
baseline value determined 30 s prior to the sample injection and a
measurement taken at 30 s post-injection. Regeneration of the
15 surface was accomplished with one 12- μ L pulse of 3 M MgCl₂.

The instrument noise level is 20 RU; therefore, any binding
activity with a signal above 20 RU may be interpreted as a real
interaction with the receptor. For C2C12-ras conditioned media, the
binding activities were in the range 60-90 RU for the TIE-2 RB
20 immobilized surface. For the same samples assayed on a EHK-1 RB
immobilized surface, the measured activities were less than 35 RU.
Specific binding to the TIE-2 receptorbody was evaluated by incubating
the samples with an excess of either soluble TIE-2 or EHK-1 RB prior
to assaying the binding activity. The addition of soluble EHK-1 RB had
25 no effect on the TIE-2 binding activity of any of the samples, while in
the presence of soluble TIE-2 binding to the surface is two-thirds less
than that measured in the absence of TIE-2. A repeat assay using >50x

concentrated C2C12-ras CCM resulted in a four-fold enhancement over background of the TIE-2 specific binding signal.

EXAMPLE 5 - C2C12-ras CCM CONTAINS AN ACTIVITY THAT
5 INDUCES TYROSINE PHOSPHORYLATION OF TIE-2
RECEPTOR

C2C12-ras 10X CCM was examined for its ability to induce tyrosine phosphorylation of TIE-2 in ABAE cells. Serum-starved ABAE 10 cells were briefly incubated with C2C12-ras CCM, lysed and subjected to immunoprecipitation and Western analyses as described above. Stimulation of serum-starved ABAE cells with serum-free C2C12-ras 15 10X CCM was done as follows. The medium of ABAE cells starved as described above was removed and replaced with either defined medium or 10X CCM that had been pre-warmed to 37°C. After 10 minutes, the media were removed and the cells were twice rinsed on ice with an excess of chilled PBS supplemented with orthovanadate/NaF/benzamidine. Cell lysis and TIE-2-specific 20 immunoprecipitation was done as described above.

ABAE cells incubated for 10 minutes with defined medium showed no induction of TIE-2 tyrosine phosphorylation, whereas 25 incubation with C2C12-ras CCM stimulated at least a 100 X increase in TIE-2 phosphorylation. This activity was almost totally depleted by pre-incubation of the C2C12-ras 10X CCM for 90 minutes at room temperature with 13 µg of TIE-2 RB coupled to protein G-Sepharose beads. Medium incubated with protein G Sepharose alone was not depleted of this phosphorylating activity.

EXAMPLE 6 - EXPRESSION CLONING OF TIE-2 LIGAND

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 1% each of penicillin and streptomycin (P/S) and 2 mM glutamine in an atmosphere of 5% CO₂. The mouse myoblast C2C12 ras cell line was cultured in Eagle's minimal essential medium (EMEM) with 10% FBS, (P/S) and 2 mM glutamine. Full length mouse TIE-2 ligand cDNA clones were obtained by screening a C2C12 ras cDNA library in the pJFE14 vector expressed 10 in COS cells. This vector, as shown in Figure 2, is a modified version of the vector pSR_α (Takebe, et al. 1988, Mol. Cell. Biol. 8:466-472). The library was created using the two BSTX1 restriction sites in the pJFE14 vector.

COS-7 cells were transiently transfected with either the pJFE14 library or control vector by the DEAE-dextran transfection protocol. Briefly, COS-7 cells were plated at a density of 1.0 x 10⁶ cells/100 mm plate 24 hours prior to transfection. For transfection, the cells were cultured in serum-free DMEM containing 400 µg/ml of DEAE-dextran, 1 µM chloroquine, and 2 mM glutamine, and 1 µg of the appropriate DNA for 3-4 hours at 37°C in an atmosphere of 5% CO₂. The transfection media was aspirated and replaced with PBS with 10% DMSO for 2-3 min. Following this DMSO "shock", the COS-7 cells were placed into DMEM with 10% FBS, 1% each of penicillin and streptomycin, and 2 mM glutamine for 48 hours.

Because the TIE-2 ligand is secreted it was necessary to permeabilize the cells to detect binding of the receptorbody probe to the ligand. Two days after transfection the cells were rinsed with PBS and then incubated with PBS containing 1.8% formaldehyde for 15-

30 min. at room temperature. Cells were then washed with PBS and incubated for 15 min. with PBS containing 0.1% Triton X-100 and 10% Bovine Calf Serum to permeabilize the cells and block non-specific binding sites.

5 The screening was conducted by direct localization of staining using a TIE-2 receptorbody (RB), which consisted of the extracellular domain of TIE-2 fused to the IgG1 constant region. This receptorbody was prepared as set forth in Example 2. A 100 mm dish of transfected, fixed and permeabilized COS cells was probed by incubating them for
10 30 min with TIE-2 RB. The cells were then washed twice with PBS and incubated for an additional 30 min with PBS/10% Bovine Calf Serum/anti-human IgG-alkaline phosphatase conjugate. After three PBS washes, cells were incubated in alkaline-phosphatase substrate for 30-60 min. The dish was then inspected microscopically for the
15 presence of stained cells. For each stained cell, a small area of cells including the stained cell was scraped from the dish using a plastic pipette tip and plasmid DNA was then rescued and used to electroporate bacterial cells. Single bacterial colonies resulting from the electroporation were picked and plasmid DNA prepared from these
20 colonies was used to transfect COS-7 cells which were probed for TIE-2 ligand expression as evidenced by binding to TIE-2 receptorbodies. This allowed identification of single clones coding for TIE-2 ligand. Confirmation of TIE-2 ligand expression was obtained by phosphorylation of the TIE-2 receptor using the method set forth in
25 Example 5. A plasmid clone encoding the TIE-2 ligand was deposited with the ATCC on October 7, 1994 and designated as "pJFE14 encoding TIE-2 ligand" under ATCC Accession No. 75910.

EXAMPLE 7 - ISOLATION AND SEQUENCING OF FULL LENGTH
cDNA CLONE ENCODING HUMAN TIE-2 LIGAND

A human fetal lung cDNA library in lambda gt-10 (see Figure 3)
5 was obtained from Clontech Laboratories, Inc. (Palo Alto, CA). Plaques
were plated at a density of 1.25×10^6 /20x20 cm plate, and replica
filters taken following standard procedures (Sambrook, et al.,
Molecular Cloning: A Laboratory Manual, 2nd Ed., page 8.46, Cold Spring
Harbor Laboratory, Cold Spring Harbor, New York).

10 Isolation of human tie-2 ligand clones was carried out as
follows. A 2.2 kb Xhol fragment from the deposited tie-2 ligand clone
(ATCC NO. 75910 - see Example 6 above) was labeled by random
priming to a specific activity of approximately 5×10^8 cpm/ng.
Hybridization was carried out at 65°C in hybridization solution
15 containing 0.5 mg/ml salmon sperm DNA. The filters were washed at
65°C in 2 x SSC, 0.1 % SDS and exposed to Kodak XAR-5 film overnight
at -70°C. Positive phage were plaque purified. High titre phage
lysates of pure phage were used for isolation of DNA via a Qiagen
column using standard techniques (Qiagen, Inc., Chatsworth, CA, 1995
catalog, page 36). Phage DNA was digested with EcoRI to release the
20 cloned cDNA fragment for subsequent subcloning. A lambda phage
vector containing human tie-2 ligand DNA was deposited with the
ATCC on October 26, 1994 under the designation λ gt10 encoding htie-2
ligand 1 (ATCC Accession No. 75928). Phage DNA may be subjected
25 directly to DNA sequence analysis by the dideoxy chain termination
method (Sanger, et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74: 5463-
5467).

Subcloning of the human tie-2 ligand DNA into a mammalian

expression vector may be accomplished as follows. The clone λ gt10 encoding htie-2 ligand 1 contains an EcoRI site located 490 base pairs downstream from the start of the coding sequence for the human TIE-2 ligand. The coding region may be excised using unique restriction sites upstream and downstream of the initiator and stop codons respectively. For example, an Spel site, located 70 bp 5' to the initiator codon, and a Bpu1102i (also known as Bspl) site, located 265 bp 3' to the stop codon, may be used to excise the complete coding region. This may then be subcloned into the pJFE14 cloning vector, using the XbaI (compatible to the Spel overhang) and the PstI sites (the PstI and Bpu1102i sites are both made blunt ended).

The coding region from the clone λ gt10 encoding htie-2 ligand 1 was sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The nucleotide and deduced amino acid sequence of human TIE-2 ligand from the clone λ gt10 encoding htie-2 ligand 1 is shown in Figure 4.

In addition, full length human tie-2 ligand cDNA clones were obtained by screening a human glioblastoma T98G cDNA library in the pJFE14 vector. Clones encoding human TIE-2 ligand were identified by DNA hybridization using a 2.2 kb XbaI fragment from the deposited tie-2 ligand clone (ATCC NO. 75910) as a probe (see Example 6 above). The coding region was sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). This sequence was nearly identical to that of clone λ gt10 encoding htie-2 ligand 1. As shown in Figure 4, the clone λ gt10 encoding htie-2 ligand 1 contains an additional glycine residue which is encoded by nucleotides 1114-1116. The coding sequence of

the T98G clone does not contain this glycine residue but otherwise is identical to the coding sequence of the clone λ gt10 encoding htie-2 ligand 1. Figure 5 sets forth the nucleotide and deduced amino acid sequence of human TIE-2 ligand from the T98G clone.

5

EXAMPLE 8 - ISOLATION AND SEQUENCING OF SECOND FULL LENGTH cDNA CLONE A ENCODING HUMAN TIE-2 LIGAND

10 A human fetal lung cDNA library in lambda gt-10 (see Figure 3) was obtained from Clontech Laboratories, Inc. (Palo Alto, CA). Plaques were plated at a density of 1.25×10^6 /20x20 cm plate, and replica filters taken following standard procedures (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., page 8.46, Cold Spring 15 Harbor Laboratory, Cold Spring Harbor, New York). Duplicate filters were screened at low stringency (2 x SSC, 55° C) with probes made to the human TIE-2 ligand 1 sequence. One of the duplicate filters was probed with a 5' probe, encoding amino acids 25 - 265 of human TIE-2 ligand 1 as set forth in Figure 4. The second duplicate filter was 20 probed with a 3' probe, encoding amino acids 282 - 498 of human TIE-2 ligand 1 sequence (see Figure 4). Both probes were hybridized at 55° C in hybridization solution containing 0.5 mg/ml salmon sperm DNA. Filters were washed in 2 x SSC at 55° C and exposed overnight to X-ray film. In addition, duplicate filters were also hybridized at normal 25 stringency (2 x SSC, 65° C) to the full length coding probe of mouse TIE-2 ligand 1 (F3-15, Xhol insert). Three positive clones were picked that fulfilled the following criteria: i. hybridization had not been seen to the full length (mouse) probe at normal stringency, and ii.

hybridization was seen at low stringency to both 5' and 3' probes.

EcoRI digestion of phage DNA obtained from these clones indicated two independent clones with insert sizes of approximately 2.2kb and approximately 1.8 kb. The 2.2kb EcoRI insert was subcloned into the

5 EcoRI sites of both pBluescript KS (Stratagene) and a mammalian expression vector suitable for use in COS cells. Two orientations were identified for the mammalian expression vector. The 2.2kb insert in pBluescript KS was deposited with the ATCC on December 9, 1994 and designated as pBluescript KS encoding human TIE 2 ligand 2. The start 10 site of the TIE-2 ligand 2 coding sequence is approximately 355 base pairs downstream of the pBluescript EcoRI site.

COS-7 cells were transiently transfected with either the expression vector or control vector by the DEAE-dextran transfection protocol. Briefly, COS-7 cells were plated at a density of 1.0×10^6

15 cells/100 mm plate 24 hours prior to transfection. For transfection, the cells were cultured in serum-free DMEM containing 400 μ g/ml of DEAE-dextran, 1 μ M chloroquine, and 2 mM glutamine, and 1 μ g of the appropriate DNA for 3-4 hours at 37°C in an atmosphere of 5% CO₂.

The transfection media was aspirated and replaced with phosphate-

20 buffered saline with 10% DMSO for 2-3 min. Following this DMSO "shock", the COS-7 cells were placed into DMEM with 10% FBS, 1% each of penicillin and streptomycin, and 2 mM glutamine for 48 hours.

Because the TIE-2 ligand is secreted it was necessary to permeabilize the cells to detect binding of the receptorbody probe to

25 the ligand. Transfected COS-7 cells were plated at a density of 1.0×10^6 cells/100 mm plate. The cells were rinsed with PBS and then incubated with PBS containing 1.8% formaldehyde for 15-30 min. at room temperature. Cells were then washed with PBS and incubated for

15 min. with PBS containing 0.1% Triton X-100 and 10% Bovine Calf Serum to permeabilize the cells and block non-specific binding sites. The screening was conducted by direct localization of staining using a TIE-2 receptorbody, which consisted of the extracellular domain of 5 TIE-2 fused to the IgG1 constant region. This receptorbody was prepared as set forth in Example 2. Transfected COS cells were probed by incubating them for 30 min with TIE-2 receptorbody. The cells were then washed twice with PBS, fixed with methanol, and then incubated for an additional 30 min with PBS/10% Bovine Calf 10 Serum/anti-human IgG-alkaline phosphatase conjugate. After three PBS washes, cells were incubated in alkaline-phosphatase substrate for 30-60 min. The dish was then inspected microscopically for the presence of stained cells. Cells expressing one orientation of the clone, but not the other orientation, were seen to bind the TIE-2 15 receptorbody.

One of skill in the art will readily see that the described methods may be used to further identify other related members of the TIE ligand family.

The coding region from the clone pBluescript KS encoding human 20 TIE-2 ligand 2 was sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The nucleotide and deduced amino acid sequence of human TIE-2 ligand from the clone pBluescript KS encoding human TIE-2 ligand 2 is shown in Figure 6.

25

EXAMPLE 9 - TIE-2 LIGAND 2 IS A RECEPTOR ANTAGONIST

Conditioned media from COS cells expressing either TIE-2 ligand 2 (TL2) or TIE-2 ligand 1 (TL1) were compared for their ability to activate TIE-2 receptors naturally present in human endothelial cell lines.

5 Lipofectamine reagent (GIBCO-BRL, Inc.) and recommended protocols were used to transfect COS-7 cells with either the pJFE14 expression vector alone, pJFE14 vector containing the human TIE-2 ligand 1 cDNA, or with a pMT21 expression vector (Kaufman, R.J., 1985, Proc. Natl. Acad. Sci. USA 82: 689-693) containing the human TIE-2
10 ligand 2 cDNA. COS media containing secreted ligands were harvested after three days and concentrated 20-fold by diafiltration (DIAFLO ultrafiltration membranes, Amicon, Inc.). The quantity of active TIE-2 ligand 1 and TIE-2 ligand 2 present in these media was determined and expressed as the amount (in resonance units, R.U.) of TIE-2 receptor
15 specific binding activity measured by a BIACore binding assay.

Northern (RNA) analyses revealed significant levels of TIE-2 transcripts in HAEC (Human Aortic Endothelial Cell) human primary endothelial cells (Clonetics, Inc.). Therefore, these cells were used to examine whether TIE-2 receptor is tyrosine-phosphorylated when
20 exposed to COS media containing the TIE-2 ligands. HAEC cells were maintained in a complete endothelial cell growth medium (Clonetics, Inc.) that contained 5% fetal bovine serum, soluble bovine brain extract, 10 ng/ml human EGF, 1 mg/ml hydrocortisone, 50 mg/ml gentamicin and 50 ng/ml amphotericin-B. Assessment of whether TL1
25 and TL2 could activate TIE-2 receptor in the HAEC cells was done as follows. Semi-confluent HAEC cells were serum-starved for two hours in high-glucose Dulbecco's MEM with added L-glutamine and penicillin-streptomycin at 37°C followed by replacement of the

starvation medium with ligand-containing conditioned COS media for 7 minutes at 37°C in a 5% CO₂ incubator. The cells were subsequently lysed and TIE-2 receptor protein was recovered by immunoprecipitation of the lysates with TIE-2 peptide antiserum, 5 followed by Western blotting with antiphosphotyrosine antiserum, exactly as described in example 1. The results are shown in Figure 7. Phosphotyrosine levels on the TIE-2 receptor (TIE-2-R) were induced by treatment of HEAC cells with TIE-2 ligand 1 (Lane L1) but not by TIE-2 ligand 2 (Lane L2) conditioned COS media. MOCK is conditioned 10 media from COS transfected with JFE14 empty vector.

Evidence that both TL1 and TL2 specifically bind to the TIE-2 receptor was demonstrated by using a BIACore to assay the TIE-2 receptor specific binding activities in transfected COS media and by immunostaining of TL1- and TL2-expressing COS cells with TIE-2 15 receptorbodies.

Because TL2 did not activate the TIE-2 receptor, applicants set out to determine whether TL2 might be capable of serving as an antagonist of TL1 activity. HAEC phosphorylation assays were performed in which cells were first incubated with an "excess" of TL2, 20 followed by addition of dilute TL1. It was reasoned that prior occupancy of TIE-2 receptor due to high levels of TL2 might prevent subsequent stimulation of the receptor following exposure to TL1 present at a limiting concentration.

Semi-confluent HAEC cells were serum-starved as described 25 above and then incubated for 3 min., at 37°C with 1-2 ml. of 20X COS/JFE14-TL2 conditioned medium. Control plates were treated with 20X COS/JFE14-only medium (MOCK). The plates were removed from the incubator and various dilutions of COS/JFE14-TL1 medium were

then added, followed by further incubation of the plates for 5-7 min. at 37°C. Cells were subsequently rinsed, lysed and TIE-2-specific tyrosine phosphorylation in the lysates was examined by receptor immunoprecipitation and Western blotting, as described above. TL1 dilutions were made using 20X COS/JFE14-TL1 medium diluted to 2X, 0.5X, 0.1X, or 0.02X by addition of 20X COS/JFE14-alone medium. An assay of the initial 20X TL1 and 20X TL2 COS media using BIACore biosensor technology indicated that they contained similar amounts of TIE-2-specific binding activities, i.e., 445 R.U. and 511 R.U. for TL1 and TL2, respectively. The results of the antiphosphotyrosine Western blot, shown in Figure 8, indicate that when compared to prior treatment of HAEC cells with MOCK medium (lane 1), prior treatment of HAEC cells with excess TIE-2 ligand 2 (lane 2) antagonizes the subsequent ability of dilute TIE-2 ligand 1 to activate the TIE-2 receptor (TIE-2-R).

The ability of TL2 to competitively inhibit TL1 activation of the TIE-2-R was further demonstrated using the human cell hybrid line, EA.hy926 (see Example 21 for detailed description of this cell line and its maintenance). Experiments were performed in which unconcentrated COS cell media containing TL1 were mixed at varying dilutions with either MOCK- or TL2- conditioned media and placed on serum-starved EA.hy926 cell monolayers for 5 minutes at 37°C. The media were then removed, the cells were harvested by lysis and TIE-2-specific tyrosine phosphorylation was examined by Western blots, as described above. Figure 9 shows an experiment which contains three groups of treatments, as viewed from left to right. As shown in the four lanes at the left, treatment of the EA.hy926 cells with 1x COS-TL1 alone robustly activated the endogenous TIE-2-R in these cells,

whereas 1x TL2 COS medium was inactive. However, mixture of TL1 with either MOCK or TL2 demonstrated that TL2 can block the activity of TL1 in a dose-dependent fashion. In the central three pairs of lanes the ratio of TL2 (or MOCK) was decreased while the amount of TL1 in 5 the mixture was correspondingly increased from 0.1x to 0.3x. At any of these mixture ratios the TL1:TL2 lanes showed a reduced level of TIE-2-R phosphorylation compared to that of the corresponding TL1:MOCK lanes. When the amount TL1 was held steady and the amount of TL2 (or MOCK) was decreased, however (shown in the three pairs of 10 lanes at the right), a point was reached at which the TL2 in the sample was too dilute to effectively inhibit TL1 activity. The relative amount of each ligand present in these conditioned COS media could be estimated from their binding units as measured by the BIACore assay and from Western blots of the COS media with ligand-specific 15 antibodies. Consequently, we can infer that only a few-fold molar excess of TL2 is required to effectively block the activity of TL1 in vitro. This is significant because we have observed distinct examples in vivo (see Example 17 and Figure 16) where TL2 mRNAs achieve considerable abundance relative to those of TL1. Thus, TL2 may be 20 serving an important physiological role in effectively blocking signaling by the TIE-2-R at these sites.

Taken together these data confirm that, unlike TL1, TL2 is unable to stimulate endogenously expressed TIE-2-R on endothelial cells. Furthermore, at a few fold molar excess TL2 can block TL1 stimulation 25 of the TIE-2 receptor, indicating that TL2 is a naturally occurring TIE-2 receptor antagonist.

EXAMPLE 10 - IDENTIFICATION OF TIE-2-SPECIFIC BINDING ACTIVITY
IN CONDITIONED MEDIUM AND COS CELL

SUPERNATANTS

5 Binding activity of 10x CCM from the cell lines C2C12-ras, Rat2
ras, SHEP, and T98G, or COS cell supernatants after transfection with
either human TIE-2 ligand 1 (hTL1) or human TIE-2 ligand 2 (hTL2) was
measured using biosensor technology (BIAcore; Pharmacia Biosensor,
Piscataway, NJ) which monitors biomolecular interactions in real-
10 time via surface plasmon resonance (SPR). Purified rat or human TIE-2
RB was covalently coupled through primary amines to the
carboxymethyl dextran layer of a CM5 research grade sensor chip
(Pharmacia Biosensor; Piscataway, NJ). The sensor chip surface was
activated using a mixture of N-hydroxysuccinimide (NHS) and N-ethyl-
15 N'-(3- dimethylaminopropyl)carbodiimide (EDC), followed by
immobilization of TIE-2 RB (25 µg/mL, pH 4.5) and deactivation of
unreacted sites with 1.0 M ethanolamine (pH 8.5). In general, 9000-
10000 RU of each receptorbody was coupled to the sensor chip.

20 The running buffer used in the system was HBS (10 mM Hepes,
150 mM NaCl, 0.005% P20 surfactant, pH 7.4). The samples were
centrifuged for 15 min at 4°C and further clarified using a sterile, low
protein-binding 0.45 µm filter (Millipore; Bedford, MA). Dextran
(2mg/ml) and P20 surfactant (0.005%) were added to each sample.
25 Aliquots of 40 µL were injected across the immobilized surface
(either rat or human TIE-2) at a flow rate of 5 µL/min and the receptor
binding was monitored for 8 min. The binding activity (resonance
units, RU) was measured as the difference between a baseline value
determined 30 s prior to the sample injection and a measurement

taken at 30 s post-injection. Regeneration of the surface was accomplished with one 15- μ L pulse of 3 M MgCl₂.

The CCM samples (C2C12-ras, Rat2-ras, SHEP, T98G) were tested on the rat TIE-2 RB immobilized surface, while the recombinant hTL1 and hTL2 were tested on the human TIE-2 RB immobilized surface. In each case, specific binding to the TIE-2 receptorbody was evaluated by incubating the samples with 25 μ g/ml of either soluble TIE-2 (rat or human) RB or trkB RB prior to assaying the binding activity. As shown in Figures 10 and 11, the addition of soluble trkB RB causes a slight decrease in the TIE-2 binding activity, while the addition of soluble TIE-2 RB significantly reduces the binding activity as compared to that measured in the absence of TIE-2 RB.

EXAMPLE 11 - TIE-2 RB SPECIFICALLY BLOCKS ACTIVATION OF THE
15 TIE-2 RECEPTOR BY TIE-2 LIGAND 1

The applicants sought to determine whether soluble TIE-2 RB can serve as a competitive inhibitor to block activation of TIE-2 receptor by TIE-2 ligand 1 (TL1). To do this, TL1-containing COS media were preincubated with either TIE-2- or TrkB-RB and then compared for their ability to activate TIE-2 receptors naturally present in a human endothelial cell line.

Conditioned COS media were generated from COS-7 cells transfected with either the pJFE14 expression vector alone (MOCK), or pJFE14 vector containing the human TIE-2 ligand 1 cDNA (TL1) and harvested as described in Example 9 hereinabove, with the exception that the media were sterile filtered but not concentrated. The quantity of TL1 was determined and expressed as the amount (in resonance

units, R.U.) of TIE-2 receptor-specific binding activity measured by BIACore binding assay.

Northern (RNA) analyses revealed significant levels of tie-2 transcripts in HUVEC (Human Umbilical Vein Endothelial Cell) human primary endothelial cells (Clonetics, Inc.). Therefore, these cells were used to examine whether TIE-2 receptor can be tyrosine-phosphorylated when exposed in the presence of TIE-2- or TrkB-RBs to COS media containing TL1. HUVEC cells were maintained at 37°C, 5% CO₂ in a complete endothelial cell growth medium (Clonetics, Inc.) that contained 5% fetal bovine serum, soluble bovine brain extract with 10 µg/ml heparin, 10 ng/ml human EGF, 1 µg/ml hydrocortisone, 50 µg/ml gentamicin and 50 ng/ml amphotericin-B. Assessment of whether TL1 could activate TIE-2 receptor in the HUVEC cells was done as follows. Confluent dishes of HUVEC cells were serum-starved for two-to-four hours in low-glucose Dulbecco's MEM at 37°C, 5% CO₂, followed by 10 minute incubation in starvation medium that included 0.1 mM sodium orthovanadate, a potent inhibitor of phosphotyrosine phosphatases. Meanwhile, conditioned COS media were preincubated 30 min. at room temperature with either TIE-2- or TrkB-RB added to 50 µg/ml. The starvation medium was then removed from the HUVEC dishes and incubated with the RB-containing COS media for 7 minutes at 37°C. HUVEC cells were subsequently lysed and TIE-2 receptor protein was recovered by immunoprecipitation with TIE-2 peptide antiserum, followed by Western blotting with an anti-phosphotyrosine antibody, as described in Example 1. The results are shown in Figure 12. Phosphotyrosine levels on the TIE-2 receptor were induced by treatment of HUVEC cells with TIE-2 ligand 1 (TL1) relative to that seen with control medium (MOCK) and this induction is specifically

blocked by prior incubation with TIE-2-RB (TIE-2-Fc) but not by incubation with TrkB-RB (TrkB-Fc). These data indicate that soluble TIE-2 RB can serve as a selective inhibitor to block activation of TIE-2 receptor by TIE-2 ligand 1.

5

EXAMPLE 12 - CONSTRUCTION OF TIE-2 LIGANDBODIES

An expression construct was created that would yield a secreted protein consisting of the entire coding sequence of human TIE-2 ligand 1 (TL1) or TIE-2 ligand 2 (TL2) fused to the human immunoglobulin gamma-1 constant region (IgG1 Fc). These fusion proteins are called TIE-2 "liganbodies" (TL1-Fc or TL2-Fc). The Fc portion of TL1-Fc and TL2-Fc was prepared as follows. A DNA fragment encoding the Fc portion of human IgG1 that spans from the hinge region to the carboxy-terminus of the protein, was amplified from human placental cDNA by PCR with oligonucleotides corresponding to the published sequence of human IgG1; the resulting DNA fragment was cloned in a plasmid vector. Appropriate DNA restriction fragments from a plasmid encoding full-length TL1 or TL2 and from the human IgG1 Fc plasmid were ligated on either side of a short PCR-derived fragment that was designed so as to fuse, in-frame, TL1 or TL2 with human IgG1 Fc protein-coding sequences.

Milligram quantities of TL2-Fc were obtained by cloning the TL2-Fc DNA fragment into the pVL1393 baculovirus vector and subsequently infecting the *Spodoptera frugiperda* SF-21AE insect cell line. Alternatively, the cell line SF-9 (ATCC Accession No. CRL-1711) or the cell line BTI-TN-5b1-4 may be used. DNA encoding the TL2-Fc was cloned as an Eco RI-NotI fragment into the baculovirus transfer

plasmid pVL1393. Plasmid DNA was recombined into viral DNA by mixing 3 µg of plasmid DNA with 0.5 µg of Baculo-Gold DNA (Pharminigen), followed by introduction into liposomes using 30µg Lipofectin (GIBCO-BRL). DNA-liposome mixtures were added to SF-5 21AE cells (2x 106 cells/60mm dish) in TMN-FH medium (Modified Grace's Insect Cell Medium (GIBCO-BRL) for 5 hours at 27°C, followed by incubation at 27°C for 5 days in TMN-FH medium supplemented with 5% fetal calf serum. Tissue culture medium was harvested for plaque purification of recombinant viruses, which was carried out using 10 methods previously described (O'Reilly, D.R., L.K. Miller, and V.A. Luckow, *Baculovirus Expression Vectors - A Laboratory Manual*, 1992, New York: W.H. Freeman) except that the agarose overlay contained 125 mg/mL X-gal (5-bromo-4-chloro-3-indolyl-b- D-galactopyranoside; GIBCO-BRL). After 5 days of incubation at 27°C, non-recombinant 15 plaques were scored by positive chromogenic reaction to the X-gal substrate, and their positions marked. Recombinant plaques were then visualized by addition of a second overlay containing 100 mg/mL MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide; Sigma). Putative recombinant virus plaques were picked by plug aspiration, and 20 purified by multiple rounds of plaque isolation to assure homogeneity. Virus stocks were generated by serial, low-multiplicity passage of plaque-purified virus. Low passage stocks of one virus clone (vTL2-Fc Clone #7) were produced.

SF-21AE cells were cultured in serum-free medium (SF-900 II, 25 Gibco BRL) containing 1X antibiotic/antimycotic solution (Gibco BRL) and 25 mg/L Gentamycin (Gibco BRL). Pluronic F-68 was added as a surfactant to a final concentration of 1g/L. Cultures (4L) were raised in a bioreactor (Artisan Cell Station System) for at least three days

prior to infection. Cells were grown at 27°C, with gassing to 50 % dissolved oxygen, at a gas flow rate of 80 mL/min (aeration at a sparge ring). Agitation was by means of a marine impeller at a rate of 100 rpm. Cells were harvested in mid-logarithmic growth phase (~2 5 X10⁶ cells/mL), concentrated by centrifugation, and infected with 5 plaque forming units of vTL2-Fc per cell. Cells and inoculum were brought to 400mL with fresh medium, and virus was adsorbed for 2 hours at 27°C in a spinner flask. The culture was then resuspended in a final volume of 8L with fresh serum-free medium, and the cells 10 incubated in the bioreactor using the previously described conditions.

Culture medium from vTL2-Fc-infected SF21AE cells were collected by centrifugation (500x g, 10 minutes) at 72 hours post-infection. Cell supernatants were brought to pH 8 with NaOH. EDTA was added to a final concentration of 10 mM and the supernatant pH 15 was readjusted to 8. Supernatants were filtered (0.45 µm, Millipore) and loaded on a protein A column (protein A sepharose 4 fast flow or HiTrap protein A, both from Pharmacia). The column was washed with PBS containing 0.5 M NaCl until the absorbance at 280 nm decreased to baseline. The column was washed in PBS and eluted with 0.5 M acetic acid. Column fractions were immediately neutralized by eluting into 20 tubes containing 1 M Tris pH 9. The peak fractions containing the TL2-Fc were pooled and dialyzed versus PBS.

EXAMPLE 13 - EXPRESSION OF TIE-1, TIE-2, TL1, AND TL2 IN RENAL
25 CELL CARCINOMA

In situ hybridization experiments were performed on human renal cell carcinoma tumor tissue using TIE-1, TIE-2, TL1, and TL2 cDNA

probes. TIE-2, TIE-1, TL1, and TL2 expression were all up-regulated in the tumor vasculature. Ligand expression appeared to be localized to either the vascular endothelial cells (TL2) or very near the vascular endothelial cells in the mesenchyme (TL1). VEGF has been shown to be 5 dramatically up-regulated in this tumor tissue. Brown, et al. Am. J. Pathol. 143:1255-1262 (1993).

10 EXAMPLE 14 - EXPRESSION OF TIE-1, TIE-2, TL1, AND TL2 IN WOUND
HEALING

15 *In situ* hybridization experiments were performed on cross-sectional tissue slices obtained from a rat cutaneous wound model using TIE-1, TIE-2, TL1, and TL2 cDNA probes. The wound healing model involves pressing a small cork bore against the skin of a rat and removing a small, cylindrical plug of skin. As healing begins at the base of the wound, a vertical slice of tissue is taken and used for *in* situ hybridization. In the tested tissue sample, TL1 and TL2 appeared 20 to be slightly up-regulated by four days post-injury. In contrast to the slightly up-regulated expression of TL1 and TL2 in this tissue, VEGF expression, which may precede TL1 and TL2 expression, is dramatically up-regulated.

25

EXAMPLE 15 - EXPRESSION OF TIE LIGANDS IN FETAL LIVER AND
THYMUS

Reverse transcription-PCR (RT-PCR) was performed on mouse E14.5 fetal liver and mouse E17.5 fetal thymus. Agarose gel electrophoresis of the RT-PCR products revealed that in the mouse fetal liver, TIE-2 ligand 1 (TL1) RNA is enriched in the stromal region, 5 but is absent in c-kit⁺TER119 hematopoietic precursor cells. In this same tissue, TIE-2 ligand 2 (TL2) RNA is enriched in the stromal cells, but absent in the hematopoietic precursor cells (Figure 13). In the mouse fetal thymus, TL2 is enriched in the stromal cells (Figure 14).

10 EXAMPLE 16 - THE TIE RECEPTOR/LIGAND SYSTEM IN ANGIOGENESIS

Although the TIE-2/TIE ligand system appears to play an important role in endothelial cell biology, it has not been shown to play a significant, active role in the early to intermediate stages of 15 vascularization (e.g. angioblast or endothelial cell proliferation and migration, tubule formation, and other early stage events in vascular modeling). In contrast to the receptors and factors known to mediate these aspects of vascular development, the temporally late pattern of expression of TIE-2 and TL1 in the course of vascularization suggests 20 that this system plays a distinct role in the latter stages vascular development, including the structural and functional differentiation and stabilization of new blood vessels. The pattern of expression of TIE-2/TL1 also is consistent with a continuing role in the maintenance 25 of the structural integrity and/or physiological characteristics of an established vasculature.

TIE Ligand 2 (TL2) appears to be a competitive inhibitor of TL1. The spatiotemporal characteristics of TL2 expression suggest that this single inhibitory molecule may play multiple, context-dependent

roles essential to appropriate vascular development or remodeling (e.g., de-stabilization/de-differentiation of mature endothelial cells allowing the formation of new vessels from existing vasculature, inhibition of inappropriate blood vessel formation, and regression/involution of mature blood vessels). Figure 15 is a schematic representation of the hypothesized role of the TIE-2/TIE ligands in angiogenesis. In this figure TL1 is represented by (•), TL2 is represented by (*), TIE-2 is represented by (T), VEGF is represented by ([]), and flk-1 (a VEGF receptor) is represented by (Y).

10

EXAMPLE 17 - EXPRESSION OF TIE LIGANDS IN THE FEMALE
REPRODUCTIVE SYSTEM: EXPRESSION IN THE
OVARY

15 Preliminary observations made in experiments examining the expression of the TIE receptors and ligands in the female reproductive system are consistent with the hypothesis the TL1 plays a role in neovascularization which temporally follows that of VEGF. The pattern of TL2 expression is also consistent with an antagonism of the 20 action of TL1, and a specific role in vascular regression. To verify this, expression of relevant mRNAs can be examined following experimental induction of follicular and luteal development so that their temporal relation to various aspects of neovascularization/vascular regression can be more clearly defined 25 (e.g. in conjunction with endothelial cell staining, vascular fills). Angiogenesis associated with follicular development and corpus luteum formation in staged ovaries of mature, female rats or following induced ovulation in pre-pubertal animals was followed

using *in situ* hybridization. Figure 16 contains photographs of *in situ* hybridization slides showing the temporal expression pattern of TIE-2, TL1, TL2, and VEGF during the ovarian cycle [Column 1: Early pre-ovulatory follicle; Column 2: pre-ovulatory follicle; Column 3: early 5 corpus luteum; and Column 4: atretic follicle; Row A: bright field; Row B: VEGF; Row C: TL2; Row D: TL1 and Row E: TIE-2 receptor]. These studies revealed that VEGF, TL1 and TL2 are expressed in a temporally and spatially coordinate fashion with respect to the development and regression of vasculature in the ovary, specifically with respect to the 10 establishment of the vascular system which is generated in the course of the conversion of an ovarian follicle to a corpus luteum (CL).

Briefly, VEGF expression increases in the follicular granule layer prior to its vascularization during the process of luteinization. During the process of CL formation, highest levels of VEGF expression are 15 apparent in the center of the developing CL in the vicinity of luteinizing cells which are not yet vascularized. VEGF levels remain moderately high and are diffusely distributed in the developed CL. In contrast, noticeably enhanced expression of TIE-2 ligand 1 occurs only late in process of CL formation, after a primary vascular plexus has 20 been established. Later, TL1 expression is apparent throughout the CL at which time the definitive capillary network of the CL has been established.

TL2 exhibits a more complex pattern of expression than either VEGF or TL1. In the developing CL, TL2 is expressed at highest levels 25 at the front of the developing capillary plexus- between the central avascular region of the CL where VEGF expression is highest, and the most peripheral portion of the CL where TL1 expression is dominant and where the luteinization process is complete and the vascular

system is most mature. TL2 also appears to be expressed at high levels in the follicular layer of large follicles which are undergoing atresia. While TL1 is also apparent in atretic follicles, VEGF is not expressed.

5 The pattern of expression described above is most consistent with a role for VEGF in the initiation of angiogenesis, with TL1 acting late in this process-for example in modeling and/or stabilization of the definitive vascular network. In contrast, TL2 is present both in areas of active expansion of a newly forming vascular network (during 10 CL formation), and in regions which fail to establish a new vasculature and vascular regression is in progress (atretic follicles). This suggests a more dynamic and complex role for TL2, possibly involving destabilization of existing vasculature (necessary for regression) or developing vasculature (necessary for the dynamic modeling of newly 15 forming vessels).

EXAMPLE 18 - A RECEPTORBODY BINDING ASSAY AND A LIGAND
BINDING AND COMPETITION ASSAY

20 A quantitative cell-free binding assay with two alternate formats has been developed for detecting either TIE-2 receptorbody binding or ligand binding and competition. In the receptorbody binding version of the assay, TIE-2 ligands (purified or partially purified; 25 either TL1 or TL2) are coated onto an ELISA plate. Receptorbody at varying concentrations is then added, which binds to the immobilized ligand in a dose-dependent manner. At the end of 2 hours, excess receptorbody is washed away, then the amount bound to the plate is

reported using a specific anti-human Fc antibody which is alkaline phosphatase tagged. Excess reporter antibody is washed away, then the AP reaction is developed using a colored substrate. The assay is quantitated using a spectrophotometer. Figure 19 shows a typical TIE-2-IgG binding curve. This assay has been used to evaluate the integrity of TIE-2-IgG after injection into rats and mice. The assay can also be used in this format as a ligand competition assay, in which purified or partially-purified TIE ligands compete with immobilized ligand for receptorbody. In the ligand binding and competition version of the binding assay, TIE-2 ectodomain is coated onto the ELISA plate. The Fc-tagged fibrinogen-like domain fragments of the TIE ligands (TL1-fFc and TL2-fFc) then bind to the ectodomain, and can be detected using the same anti-human Fc antibody as described above. Figure 20 shows an example of TL1-fFc binding to TIE-2 ectodomain. This version of the assay can also be used to quantitate levels of TL1-fFc in serum or other samples. If untagged ligand (again, either purified or unpurified) is added at the same time as the TL1-fFc, then a competition is set up between tagged ligand fragment and full-length ligand. The full-length ligand can displace the Fc-tagged fragment, and a competition curve is generated.

EXAMPLE 19 - EA.hy926 CELL LINE CAN BE USED AS A REPORTER
CELL LINE FOR TIE LIGAND ACTIVITY

EA.hy926 is a cell hybrid line that was established by fusion of
5 HUVEC with the human lung carcinoma-derived line, A549 [Edgell, et al.
Proc. Natl. Acad. Sci. (USA) 80, 3734-3737 (1983). EA.hy926 cells
have been found to express significant levels of TIE-2 receptor protein
with low basal phosphotyrosine levels. The density at which EA.hy926
cells are passaged prior to their use for receptor assays, as well as
10 their degree of confluence at the time of assay, can affect TIE-2
receptor abundance and relative inducibility in response to treatment
with ligand. By adopting the following regimen for growing these cells
the EA.hy926 cell line can be used as a dependable system for assay of
TIE-2 ligand activities.

15

EA.hy926 cells are seeded at 1.5×10^6 cells in T-75 flasks
(Falconware) and re-fed every other day with high-glucose Dulbecco's
MEM, 10% fetal bovine serum, L-glutamine, penicillin-streptomycin,
20 and 1x hypoxanthine-aminopterin-thymidine (HAT, Gibco/BRL). After
three to four days of growth, the cells are passaged once again at $1.5 \times$
 10^6 cells per T-75 flask and cultured an additional three to four days.
For phosphorylation assays, cells prepared as described above were
serum-starved by replacement of the culture medium with high-
25 glucose DMEM and incubation for 2-3 hours at 37°C. This medium was
aspirated from the flask and samples of conditioned media or purified
ligand were added to the flask in a total volume of 1.5 ml followed by
incubation at 37°C for 5 minutes. Flasks were removed from the

incubator and placed on a bed of ice. The medium was removed and replaced with 1.25 ml Lysis Buffer containing 1% nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS in 20 mM Tris, pH 7.6, 150 mM NaCl, 50 mM NaF, 1mM sodium orthovanadate, 5 mM benzamidine, and 1mM EDTA
5 containing the protease inhibitors PMSF, aprotinin, and leupeptin. After 10 minutes on ice to allow membrane solubilization, plates were scraped and cell lysates were clarified by microcentrifugation at top speed for 10 minutes at 4°C. TIE-2 receptor was immunoprecipitated from the clarified supernatant by incubation in the cold with an anti-
10 TIE-2 polyclonal antiserum and Protein G-conjugated Sepharose beads. The beads were washed three times with cold cell lysis buffer and boiled 5 minutes in Laemmli sample buffer, which was then loaded on 7.5% SDS-polyacrylamide gels. Resolved proteins were
15 electrotransferred to PVDF (Lamblia-P) membrane and then subjected to Western blot analysis using anti-phosphotyrosine antibody and the ECL reagent. Subsequent comparison of total TIE-2 protein levels on the same blots was done by stripping the anti-phosphotyrosine antibody and reincubating with a polyclonal antiserum specific to the ectodomain of TIE-2.
20

EXAMPLE 20 - ISOLATION AND SEQUENCING OF FULL LENGTH cDNA
CLONE ENCODING MAMMALIAN TIE LIGAND-3

25 TIE ligand-3 (TL3) was cloned from a mouse BAC genomic library (Research Genetics) by hybridizing library duplicates, with either mouse TL1 or mouse TL2 probes corresponding to the entire coding sequence of those genes. Each copy of the library was hybridized using

phosphate buffer at 55°C overnight. After hybridization, the filters were washed using 2xSSC, 0.1% SDS at 60°C, followed by exposure of X ray film to the filters. Strong hybridization signals were identified corresponding to mouse TL1 and mouse TL2. In addition, signals were 5 identified which weakly hybridized to both mouse TL1 and mouse TL2. DNA corresponding to these clones was purified, then digested with restriction enzymes, and two fragments which hybridized to the original probes were subcloned into a bacterial plasmid and sequenced. The sequence of the fragments contained two exons with homology to 10 both mouse TL1 and mouse TL2. Primers specific for these sequences were used as PCR primers to identify tissues containing transcripts corresponding to TL3. A PCR band corresponding to TL3 was identified in a mouse uterus cDNA library in lambda gt-11. (Clontech Laboratories, Inc., Palo Alto, CA).

15

Plaques were plated at a density of 1.25×10^6 /20x20 cm plate and replica filters taken following standard procedures (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., page 8.46, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). Duplicate filters 20 were screened at "normal" stringency (2 x SSC, 65°C) with a 200 bp PCR radioactive probe made to the mouse TL3 sequence. Hybridization was at 65°C in a solution containing 0.5 mg/ml salmon sperm DNA. Filters were washed in 2 x SSC at 65°C and exposed for 6 hours to X-ray film. Two positive clones that hybridized in duplicate were 25 picked. EcoRI digestion of phage DNA obtained from these clones indicated two independent clones with insert sizes of approximately 1.2 kb and approximately 2.2 kb. The 2.2kb EcoRI insert was subcloned into the EcoRI site of pBluescript KS (Stratagene). Sequence analysis

showed that the longer clone was lacking an initiator methionine and signal peptide but otherwise encoded a probe homologous to both mouse TL1 and mouse TL2.

5 Two TL3-specific PCR primers were then synthesised as follows:

US2: cctctgggctcgccagttgttagg

US1: ccagctggcagatatcagg

The following PCR reactions were performed using expression

10 libraries derived from the mouse cell lines C2C12ras and MG87. In the primary PCR reaction, the specific primer US2 was used in conjunction with vector-specific oligos to allow amplification in either orientation. PCR was in a total volume of 100ml using 35 cycles of 94° C, 1 min; 42° C or 48° C for 1 min; 72° C, 1 min. The secondary PCR
15 reaction included the second specific primer, US1, which is contained within the primary PCR product, in conjunction with the same vector oligos. The secondary reactions were for 30 cycles, using the same temperatures and times as previous. PCR products were gel isolated and submitted for sequence analysis. On the basis of sequences
20 obtained from a total of four independent PCR reactions using two different cDNA libraries, the 5' end of the TL3 sequence was deduced. Northern analysis revealed moderate to low levels of mouse TL3 transcript in mouse placenta. The expression of mouse TL3 consisted of a transcript of approximately 3 kb. The full length TL3 coding
25 sequence is set forth in Figure 21.

The mouse TL3 sequence may then be used to obtain a human clone containing the coding sequence of human TL3 by hybridizing either a

human genomic or cDNA library with a probe corresponding to mouse TL3 as has been described previously, for example, in Example 8 supra.

EXAMPLE 21 - ISOLATION OF FULL LENGTH GENOMIC CLONE ENCODING
5 HUMAN TIE LIGAND-4

TIE ligand-4 (TL4) was cloned from a mouse BAC genomic library (BAC HUMAN (II), Genome Systems Inc.) by hybridizing library duplicates, with either a human TL1 radioactive probe corresponding to the entire 10 fibrinogen coding sequence of TL1 (nucleotides 1153 to 1806 of Figure 4) or a mouse TL3 radioactive probe corresponding to a segment of 186 nucleotides from the fibrinogen region of mouse TL3 (nucleotides 1307 to 1492 of Figure 21). Each probe was labeled by PCR using exact oligonucleotides and standard PCR conditions, except that dCTP was 15 replaced by P³²dCTP. The PCR mixture was then passed through a gel filtration column to separate the probe from free P³² dCTP. Each copy of the library was hybridized using phosphate buffer, and radioactive probe at 55°C overnight using standard hybridization conditions. After hybridization, the filters were washed using 2xSSC, 0.1% SDS at 55°C, 20 followed by exposure of X ray film. Strong hybridization signals were observed corresponding to human TL1. In addition, signals were identified which weakly hybridized to both human TL1 and mouse TL3. DNA corresponding to these clones was purified using standard procedures, then digested with restriction enzymes, and one fragment 25 which hybridized to the original probes was subcloned into a bacterial plasmid and sequenced. The sequence of the fragments contained one exon with homology to both human TL1 and mouse TL3 and other members of the TIE ligand family. Primers specific for these

sequences may be used as PCR primers to identify tissues containing transcripts corresponding to TL4.

The complete sequence of human TL4 may be obtained by sequencing 5 the full BAC clone contained in the deposited bacterial cells. Exons may be identified by homology to known members of the TIE-ligand family such as TL1, TL2 and TL3. The full coding sequence of TL4 may then be determined by splicing together the exons from the TL4 genomic clone which, in turn, may be used to produce the TL4 protein. 10 Alternatively, the exons may be used as probes to obtain a full length cDNA clone, which may then be used to produce the TL4 protein. Exons may also be identified from the BAC clone sequence by homology to protein domains such as fibrinogen domains, coiled coil domains, or protein signals such as signal peptide sequences. Missing exons from 15 the BAC clone may be obtained by identification of contiguous BAC clones, for example, by using the ends of the deposited BAC clone as probes to screen a human genomic library such as the one used herein, by using the exon sequence contained in the BAC clone to screen a cDNA library, or by performing either 5' or 3' RACE procedure using 20 oligonucleotide primers based on the TL4 exon sequences.

Identification of Additional TIE Ligand Family Members

The novel TIE ligand-4 sequence may be used in a rational search for 25 additional members of the TIE ligand family using an approach that takes advantage of the existence of conserved segments of strong homology between the known family members. For example, an alignment of the amino acid sequences of the TIE ligands shows

several regions of conserved sequence (see boxed regions of Figure 22). Degenerate oligonucleotides essentially based on these boxes in combination with either previously known or novel TIE ligand homology segments may be used to identify new TIE ligands.

5

The highly conserved regions among TL1, TL2 and TL3 may be used in designing degenerate oligonucleotide primers with which to prime PCR reactions using cDNAs. cDNA templates may be generated by reverse transcription of tissue RNAs using oligo d(T) or other appropriate primers. Aliquots of the PCR reactions may then be subjected to 10 electrophoresis on an agarose gel. Resulting amplified DNA fragments may be cloned by insertion into plasmids, sequenced and the DNA sequences compared with those of all known TIE ligands.

15 Size-selected amplified DNA fragments from these PCR reactions may be cloned into plasmids, introduced into E. coli by electroporation, and transformants plated on selective agar. Bacterial colonies from PCR transformation may be analyzed by sequencing of plasmid DNAs that are purified by standard plasmid procedures.

20

Cloned fragments containing a segment of a novel TIE ligand may be used as hybridization probes to obtain full length cDNA clones from a cDNA library. For example, the human TL4 genomic sequence may be used to obtain a human cDNA clone containing the complete coding 25 sequence of human TL4 by hybridizing a human cDNA library with a probe corresponding to human TL4 as has been described previously.

EXAMPLE 22 - CLONING OF THE FULL CODING SEQUENCE OF hTL4

Both 5' and 3' coding sequence from the genomic human TL-4 clone encoding human TIE ligand-4 (hTL-4 ATCC Accession No. 98095) was 5 obtained by restriction enzyme digestion, Southern blotting and hybridization of the hTL-4 clone to coding sequences from mouse TL3, followed by subcloning and sequencing the hybridizing fragments. Coding sequences corresponding to the N-terminal and C-terminal amino acids of hTL4 were used to design PCR primers (shown below), 10 which in turn were used for PCR amplification of TL4 from human ovary cDNA. A PCR band was identified as corresponding to human TL4 by DNA sequencing using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The PCR band was then subcloned into vector pCR-script and 15 several plasmid clones were analyzed by sequencing. The complete human TL4 coding sequence was then compiled and is shown in Figure 23. In another embodiment of the invention, the nucleotide at position 569 is changed from A to G, resulting in an amino acid change from Q to R.

20

The PCR primers used as described above were designed as follows:

hTL4atg 5'-gcatgctatctcgagccaccATGCTCTCCCAGCTAGCCATGCTGCAG-
3'

25 hTL4not 5'-

gtgtcgacgcggccgcttagatcagacTTAGATGTCAAAGGCCGTATCATCAT-3'

Lowercase letters indicate "tail" sequences added to the PCR primers

to facilitate cloning of the amplified PCR fragments.

EXAMPLE 23 - CONSTRUCTION AND CHARACTERIZATION OF MODIFIED
TIE LIGANDS

5 A genetic analysis of TIE-2 ligand-1 and TIE-2 ligand-2 (TL1 and TL2) was undertaken to gain insight into a number of their observed properties. Although TL1 and TL2 share similar structural homology, they exhibit different physical and biological properties. The most 10 prominent feature that distinguishes the two ligands is that although they both bind to the TIE-2 receptor, TL1 is an agonist while TL2 is an antagonist. Under non-reducing electrophoretic conditions both proteins exhibit covalent, multimeric structures. TL1 is produced as a mixture of disulfide cross-linked multimers, primarily trimers and higher order species, without any dimeric species. But TL2 is produced 15 almost exclusively as a dimeric species. Also, while TL2 is produced well in most expression systems, TL1 is expressed poorly and is difficult to produce in large quantities. Finally, production and purification conditions also appear to predispose TL1 to inactivation by proteolytic cleavage at a site near the amino terminus.

20

To study these differences, several modified ligands were constructed as follows.

23.1. Cysteine substitution - Investigations into what factors might 25 be contributing to the different physical and biological properties of the two molecules revealed the presence in TL1 of a cysteine residue (CYS 265 in Figure 4; CYS 245 in Figure 17) preceding the fibrinogen-like domain in TL1 but absent in TL2 - i.e., there was no corresponding

cysteine residue in TL2. The CYS265 residue in TL1 is encoded by TGC and is located at about nucleotides 1102-1104 (see Figure 4) at the approximate junction between the coiled-coil and fibrinogen-like domains. Because cysteine residues are generally involved in disulfide bond formation, the presence of which can contribute to both the tertiary structure and biological properties of a molecule, it was thought that perhaps the presence of the CYS265 residue in TL1 might be at least partially responsible for the different properties of the two molecules.

10

To test this hypothesis, an expression plasmid was constructed which contained a mutation in TL1 in which the CYS (residue 265 in Figure 4; residue 245 in Figure 17) was replaced with an amino acid (serine) which does not form disulfide bonds. In addition to this TL1/CYS- mutant, a second expression plasmid was constructed which mutated the approximately corresponding position in TL2 (Met247 in Figure 17) so that this residue was now a cysteine. Both non-mutated and mutated expression plasmids of TL1 and TL2 were transiently transfected into COS7 cells, cell supernatants containing the recombinant proteins were harvested, and samples were subjected to both reducing and non-reducing SDS/PAGE electrophoresis and subsequent Western blotting.

Figure 18 shows the Western blots under non-reducing conditions of both non-mutated and mutated TL1 and TL2 proteins, revealing that the TL1/CYS- mutant runs as a dimer much like TL2 and that the TL2/CYS+ mutant is able to form a trimer, as well as higher-order multimers, more like TL1. When the two mutant proteins were tested for their

ability to induce phosphorylation in TIE-2 expressing cells, the TL1/CYS⁻ mutant was able to activate the TIE-2 receptor, whereas the TL2/CYS⁺ mutant was not.

5 Thus, when the cysteine residue (residue 265 in Figure 4; residue 245 in Figure 17) of TL1 was genetically altered to a serine, it was found that the covalent structure of TL1 became similar to that of TL2, i.e., primarily dimeric. The modified TL1 molecule still behaved as an agonist, thus the trimeric and/or higher order multimeric structure
10 was not the determining factor giving TL1 the ability to activate. Although the removal of the cysteine did make a molecule with more desirable properties, it did not improve the production level of TL1.

23.2. Domain deletions - The nucleotide sequences encoding TL1 and
15 TL2 share a genetic structure that can be divided into three domains, based on the amino acid sequences of the mature proteins. The last approximately 215 amino acid residues of each mature protein contains six cysteines and bears strong resemblance to a domain of fibrinogen. This region was thus denoted the "fibrinogen-like" domain
20 or "F-domain." A central region of the mature protein containing approximately 205 residues had a high probability of assuming a "coiled-coil" structure and was denoted the "coiled-coil" domain or "C-domain." The amino-terminal approximately 55 residues of the mature protein contained two cysteines and had a low probability of
25 having a coiled-coil structure. This region was designated the "N-terminal" domain or "N-domain." The modified ligands described herein are designated using a terminology wherein N = N-terminal domain, C = coiled-coil domain, F = fibrinogen-like domain and the

numbers 1 and 2 refer to TL1 and TL2 respectively. Thus 1N indicates the N-terminal domain from TL1, 2F indicates the fibrinogen-like domain of TL2, and so forth.

5 In order to test whether the fibrinogen-like domain (F-domain) of the TIE-2 ligands contained TIE-2 activating activity, expression plasmids were constructed which deleted the coiled-coil and N-terminal domains, leaving only that portion of the DNA sequence encoding the F-domain (for TL1, beginning in Figure 4 at about nucleotide 1159, amino acid residue ARG284; for TL2, corresponding to about nucleotide 1200 in Figure 6, amino acid residue 282). This mutant construct was then transiently transfected into COS cells. The supernatant containing the recombinant protein was harvested. The TL1/F-domain mutant was tested for its ability to bind the TIE-2 receptor. The results showed
10 that, as a monomer, the TL1/F-domain mutant was not able to bind TIE-2 at a detectable level.
15

But when the TL1/F-domain monomer was myc-tagged and subsequently clustered with an antibody directed against the myc tag,
20 it exhibited detectable binding to TIE-2. However, the antibody-clustered TL1/F-domain mutant was not able to induce phosphorylation in a TIE-2 expressing cell line.

Thus it was determined that the F-domain of the TIE-2 ligands is involved in binding the receptor but that a truncation consisting of just the F-domain alone is not sufficient for receptor binding. This raised the possibility that the coiled-coil domain was responsible for holding together several fibrinogen-like domains, which might be
25

essential for receptor binding. In an attempt to confirm this hypothesis, the F-domain was fused with the Fc section of human antibody IgG1. Because Fc sections dimerize upon expression by mammalian cells, these recombinant proteins mimicked the 5 theoretical configuration of the F-domains were the native ligands to dimerize. This F-domain-Fc construct bound but failed to activate the receptor. Apparently, multimerization caused by other regions of the ligands is necessary to enable the ligands to bind the TIE-2 receptor. In addition, some other factor outside of the F-domain must contribute 10 to phosphorylation of the receptor.

Mutants were then constructed which were missing the fibrinogen-like domain, and therefore contained only the N-terminal and coiled-coil domains. They were not capable of binding to the receptor. To assess 15 the role of the N-terminal domain in receptor binding and activation, the ligands were truncated to just their C- and F-domains and tagged with a FLAG tag at the N-terminus, creating constructs termed FLAG-1C1F and FLAG-2C2F. Although these molecules stained robustly in COS7 cells transfected transiently to express the TIE-2 receptor, they 20 failed to respond in a phosphorylation assay. Thus the N-domain does contain an essential factor for receptor activation although, as disclosed infra, the ability of chimeric molecule 2N2C1F to activate the receptor shows that even the N-domain of an inactive ligand can fill that role.

25

The differences in behavior between the myc-tagged F-domain truncation and the Fc-tagged F-domain truncation described previously suggested that the TIE ligands can only bind in dimeric or higher

multimeric forms. Indeed, non-reducing SDS-PAGE showed that the TIE ligands exist naturally in dimeric, trimeric, and multimeric forms. That the FLAG-1C1F and FLAG-2C2F truncations can bind to the TIE-2 receptor without dimerization by a synthetic tag (such as Fc), whereas 5 the F truncations cannot, suggests that the C-region is at least partly responsible for the aggregation of the F-domains.

23.3. Swapping constructs (chimeras):

Applicants had noted that the level of production of TL1 in COS7 cells 10 was approximately tenfold lower than production of TL2. Therefore, chimeras of TL1 and TL2 were constructed in an attempt to explain this difference and also to further characterize the agonist activity of TL1 as compared to the antagonist activity of TL2.

15 Four chimeras were constructed in which either the N-terminal domain or the fibrinogen domain was exchanged between TL1 and TL2 and were designated using the terminology described previously such that, for example, 1N1C2F refers to a chimera having the N-terminal and coiled-coil domains of TL1, together with the fibrinogen-like domain from 20 TL2. The four chimeras were constructed as follows:

chimera 1 -	1N1C2F
chimera 2 -	2N2C1F
chimera 3 -	1N2C2F
chimera 4 -	2N1C1F

25 The nucleotide and amino acid sequences of chimeras 1-4 are shown in Figures 24-27 respectively.

Each chimera was inserted into a separate expression vector pJFE14.

The chimeras were then transfected into COS7 cells, along with the empty pJFE14 vector, native TL1, and native TL2 as controls, and the culture supernatants were collected.

- 5 In order to determine how the swapping affected the level of expression of the ligands, a 1:5 dilution and a 1:50 dilution of the COS7 supernatants were dot-blotted onto nitrocellulose. Three ligands that contained the TL1 N-domain (i.e. native TL1, 1N2C2F and 1N1C2F) were then probed with a rabbit antibody specific to the N-terminus of TL1.
- 10 Three ligands containing the TL2 N-domain, (i.e. native TL2, 2N1C1F and 2N2C1F) were probed with a rabbit antibody specific for the N-terminus of TL2. The results demonstrated that the COS7 cells were expressing any molecule containing the N-domain of TL2 at roughly ten times the level of any molecule containing the TL1 N-domain,
- 15 regardless of the makeup of the rest of the protein. The conclusion was that the N-domain must principally control the level of expression of the ligand.

The next question addressed was the chimeras' ability or inability to activate the TIE-2 receptor. EAhy926 cells were challenged with the four chimeras, as well as TL1 as a positive control for phosphorylation and TL2 or an empty pJFE14-transfected COS7 cell supernatant as negative controls for phosphorylation. The cells were lysed, and the TIE-2 receptor was immunoprecipitated out of the cell lysate and run on an SDS-PAGE. The samples were Western blotted and probed with an anti-phosphotyrosine antibody to detect any receptors that had been phosphorylated. Surprisingly, only the constructs containing the TL1 fibrinogen-like domain (2N1C1F and 2N2C1F) could phosphorylate the

TIE-2 receptor. Thus, although the N-terminal region of TL1 is essential for activation, it can be replaced by the N-terminal region of TL2, i.e., the information that determines whether the ligand is an agonist or an antagonist is actually contained in the fibrinogen-like domain.

5 Thus it was determined that the F-domain, in addition to binding the TIE-2 receptor, is responsible for the phosphorylation activity of TL1. Further, when TL2, an otherwise inactive molecule, was altered by 10 replacing its F-domain with the TL1 F-domain, the altered TL2 acted as an agonist.

15 The 2N1C1F construct was somewhat more potent, however. The signal caused by chimera 2N1C1F appeared slightly stronger than that of chimera 2N2C1F, leading to speculation that the C-domain of TL1, though not crucial for phosphorylation, might enhance the potency of TL1. However, since the samples used for the phosphorylation assay were not normalized in terms of the concentration of ligand, it was possible that a stronger phosphorylation signal only indicated the 20 presence of more ligand. The phosphorylation assay was therefore repeated with varying amounts of ligand to determine whether the active chimeras displayed different potencies. The concentration of ligand in the COS7 supernatants of ligand transfections was determined through BIAcore biosensor technology according to methods 25 previously described (Stitt, T.N., et al. (1995) Cell 80: 661-670). BIAcore measured the binding activity of a supernatant to the TIE-2 receptor in arbitrary units called resonance units (RU). Fairly good correlation between RU's and ligand concentration has been generally

observed, with 400 RU of activity corresponding to about 1 μ g of protein per mL of supernatant. Samples were diluted to concentrations of 100 RU, 20 RU, and 5 RU each and the phosphorylation assay was repeated. The results demonstrated that chimera 2N2C1F was clearly 5 more potent than either the native TL1 or chimera 1N1C2F at the same concentrations.

Another interesting aspect of these exchange constructs is in their levels of expression. Each of the four chimeras was tested for its 10 level of production in COS cells, its ability to bind to TIE2, and its ability to phosphorylate TIE2. The results of these experiments showed that chimeras 1 and 3 were produced at levels comparable to TL1, whereas chimeras 2 and 4 were produced at levels comparable to TL2. Thus a high level of protein production was correlated with the 15 TL2 N-terminal domain. Additionally, when tested on endothelial EAhy926 cells, chimeras 2 and 4 were active, whereas 1 and 3 were not. Thus activity (phosphorylation of the receptor) correlates with the TL1 fibrinogen-like domain. Chimeras 2 and 4 therefore each had the desirable properties of high production levels as well as agonist 20 activity.

23.4. Proteolytic resistant constructs - Based on the observation that a large fraction of TL1 preparations was often proteolytically cleaved near the N-terminus, it was proposed that an arginine residue located 25 at position 49 of the mature protein (see Figure 17) was a candidate cleavage site that might be involved in the regulation of the protein's activity in vivo, and that replacing the arginine with a serine (R49->S) might increase the stability of the protein without necessarily

affecting its activity. Such a mutant of TL1 was constructed and was found to be about as active as the native TL1 but did not exhibit resistance to proteolytic cleavage.

5 23.5. Combination mutants - The most potent of the chimeric constructs, 2N1C1F, was additionally altered so that the cysteine encoded by nucleotides 784-787 as shown in Figure 27 was converted to a serine. This molecule (denoted 2N1C1F (C246S)) was expressed well, potently activated the receptor, was resistant to proteolytic
10 cleavage and was primarily dimeric, rather than higher-order multimeric. Thus the 2N domain appeared to confer protease resistance on the molecule. Finally, this molecule was further altered to eliminate the potentially protease sensitive site encoded by nucleotides 199-201 as shown in Figure 27, to give a molecule
15 (denoted 2N1C1F (R51->S,C246->S)) which was expected to be activating, well expressed, dimeric, and protease resistant.

Table 1 summarizes the modified TIE-2 ligand constructs that were made and characterizes each of them in terms of ability to bind the
20 TIE-2 receptor, ability to activate the TIE-2 receptor, the type of structure formed (monomer, dimer, etc.) and their relative production levels. Unmodified TL1 (plain) and TL2 (striped) are shown with the three domains as boxes. Thus striped boxes indicate domains from TL2. The cysteine located at position 245 of the mature TL1 protein is
25 indicated by a "C." An "X" through the "C" indicates that that cysteine residue was substituted for by another amino acid as in, for example, the TL1 CYS- mutant. Similarly, an "X" through the "R" in the last construct indicates the substitution for an Arg residue at position 49

of the mature TL1 protein. The "C" is present in one modified TL2 construct showing the TL2 CYS⁺ mutant. Constructs having Fc tails or flag tagging are also indicated.

5 Based upon the teachings herein, one of skill in the art can readily see that further constructs may be made in order to create additional modified and chimeric TIE-2 ligands which have altered properties. For example, one may create a construct comprised of the N-terminal domain of TL2 and the F-domain of TL1 fused with the Fc section of 10 human antibody IgG1. This construct would be expected to bind and activate the TIE-2 receptor. Similarly, other constructs may be created using the teachings herein and are therefore considered to be within the scope of this invention.

15 23.6. Materials and Methods -

Construction of Chimeras

Swapping constructs were inserted into a pJFE14 vector in which the XbaI site was changed to an Ascl site. This vector was then digested with Ascl and NotI yielding an Ascl-NotI backbone. DNA fragments for 20 the chimeras were generated by PCR using appropriate oligonucleotides.

The FLAG-1C1F and FLAG-2C2F inserts were subcloned into a pMT21 vector backbone that had been digested with EcoRI and NotI. The "CF" 25 truncations were obtained through PCR, and the FLAG tag and a preceding trypsin signalling sequence were constructed by annealing synthetic oligonucleotides.

Transfections

All constructs were transfected transiently into COS7 cells using either DEAE-Dextran or LipofectAMINE according to standard protocols. Cell cultures were harvested 3 days after the transfection and spun 5 down at 1000 rpm for 1 minute, and the supernatants were transferred to fresh tubes and stored at -20°C.

Staining of FLAG-1C1F-Transfected and FLAG-2C2F-Transfected Cells

6-well dishes of COS7 cells were transfected transiently with the 10 TIE-2 receptor. The COS7 supernatant from various ligand transfections was incubated on the cells for 30 minutes, followed by two washes with Phosphate Buffered Saline (PBS) without magnesium or calcium. The cells were fixed in -20°C methanol for 3 minutes, washed once with PBS, and incubated with anti-FLAG M2 antibody 15 (IBI;1:3000 dilution) in PBS/10% Bovine Calf Serum (BCS) for 30 minutes. The cells were washed once with PBS and incubated with goat anti-mouse IgG Alkaline Phosphatase (AP) conjugated antibody (Promega;1:1000) in PBS/10% BCS. The cells were washed twice with PBS and incubated with the phosphate substrate, BCIP/NBT, with 1mM 20 levamisole.

Phosphorylation Assays

Dilution of COS7 supernatants for the dose response study was done in the supernatants of COS7 cells transfected with the empty vector 25 pJFE14. EA cells that naturally express the TIE-2 receptor were starved for >2 hours in serum-free medium, followed by challenge with the appropriate COS7 supernatant for 10 minutes at 37°C in an atmosphere of 5% CO₂. The cells were then rinsed in ice-cold PBS and

lysed with 1% NP40 lysis buffer containing protease inhibitors (10 µg/ml leupeptin, 10 µg/ml aprotinin, 1mM PMSF) followed by immunoprecipitation with an antibody specific for the TIE-2 receptor. Samples were then subjected to immunoblot analysis, using anti pTyr antibodies.

5

Dot Blots

Samples were applied to a nitrocellulose membrane, which was blocked and probed with the appropriate antibodies.

10

23.7 Production of Chimeric Tie-2 Ligand from CHO and Baculovirus Infected Insect Cells

Virus Production

15 The gene for the chimeric ligand (denoted 2N1C1F (C246S)) was engineered into a baculovirus expression plasmid and recombined with viral DNA to generate recombinant baculovirus, amplified and harvested using methods previously described (O'Reilly, D.R., L.K. Miller, and V.A. Luckow, Baculovirus Expression Vectors - A Laboratory

20 Manual 1992, New York: W.H. Freeman). SF21 insect cells (Spodoptera frugiperda) obtained from Invitrogen were adapted and expanded at 27°C in Gibco SF900 II serum-free medium. Uninfected cells were grown to a density of 1x10⁶ cells/mL. Cell density was determined by counting viable cells using a hemacytometer. The virus stock for the

25 ligand was added to the bioreactor at a low multiplicity 0.01-0.1 PFU/cell to begin the infection. The infection process was allowed to continue for 3-4 days allowing maximum virus replication without incurring substantial cell lysis. The cell suspension was aseptically

aliquoted into sterile centrifuge bottles and the cells removed by centrifugation (1600 RPM, 30 min). The cell-free supernatant was collected in sterile bottles and stored at 4°C in the absence of light until further use.

5

The virus titer was determined by plaque assay as described by O'Reilly, Miller and Luckow. The method is carried out in 60mm tissue-culture dishes which are seeded with 1.5×10^6 cells. Serial dilutions of the virus stock are added to the attached cells and the mixture incubated with rocking to allow the virus to adsorb to individual cells. An agar overlay is added and plates incubated for 5 days at 27°C. Viable cells were stained with neutral red revealing circular plaques which were counted to give the virus titer expressed in plaque forming unit per milliliter (PFU/mL).

15

Infection of Cells for Protein Production

Uninfected SF21 cells were grown in tissue culture plates, and virus containing the chimeric ligand gene was added at a multiplicity of 1-10 pfu/cell. The virus was allowed to adsorb for 90 minutes at 27C with gentle rocking, after which the cells were refed with fresh amounts of Sf-900 II serum-free medium. After 3 days of growth at 27C, tissue culture fluids were harvested, and the ligand detected by immunoblotting.

25

CHO expression of Tie-2 ligand chimeras

Tie-2 ligand chimeras were cloned into any of several mammalian cell expression vectors, including (but not limited to) pJFE, pcDNA3,

pMT21, pED or others. Plasmids were transfected into CHO DG44 cells (Urlaub, G. and Chasin, L.A. 1980.. Isolation of Chinese hamster cell mutants deficient in dihydrofolate reductase activity. Proc. Natl. Acad. Sci. U.S.A. 77:4216-4220; Urlaub, G., Kas, E., Carothers, A.M., and

5 Chasin, L.A. 1983. Deletion of the diploid dihydrofolate locus from cultured mammalian cells. Cell 33:405-412) by calcium phosphate precipitation or cationic liposomes. In the case of vectors lacking a *dhfr* selectable marker, the plasmid pSV2.dhfr was cotransfected at a 20% molar ratio to the plasmid containing the TIE ligand chimera.

10 DHFR+ cells were selected by growth in selection medium (a medium lacking nucleosides and nucleotides containing 10% dialyzed fetal calf serum), and clones screened for production of chimeric TIE ligands by immunoblotting with a TIE2 receptor body. Clones expressing the desired protein were subjected to several rounds of gene amplification

15 using graded concentrations of methotrexate in selection medium. Highly expressing clones were identified after gene amplification by similar immunoblotting techniques.

Cell lines expressing chimeric TIE ligands were cultured in

20 monolayers, suspension flasks, roller bottles, and bioreactors in selection medium or in medium lacking selection, and can be grown in serum-free medium formulations.

TABLE 1
MUTATION ANALYSIS OF TIE LIGANDS

	N COILED-COIL	FIBRINOGEN-LIKE	TIE2 Binding	TIE2 Activation	Multimeric Structure	Production Levels
TL1	[]	c []	+	+	HIGHER ORDER	LOW
TL2	[]	[]	+	-	DIMER	HIGH
	[]	X []	+	+	DIMER	LOW
	[]	c []	+	-	HIGHER ORDER	HIGH
	[]	c []	-	N.D.	N.D.	LOW
	[]	[]	-	N.D.	N.D.	HIGH
	[]	[]	-	-	MONOMER	HIGH
	[]	[]	-	-	MONOMER	HIGH
	[]	Fc []	+	-	DIMER	HIGH
	[]	Fc []	+	-	DIMER	HIGH
	[]	c [] Fc []	+	+	HIGHER ORDER	LOW
	[]	[] Fc []	+	-	HIGHER ORDER	LOW
flag-	[]	c []	+	+	N.D.	LOW
flag-	[]	[]	+	-	N.D.	HIGH
flag-	[]	c []	+	-	N.D.	HIGH
flag-	[]	[]	+	-	N.D.	HIGH
	[]	c []	+	-	N.D.	LOW
	[]	[]	+	+	N.D.	HIGH
	[]	[]	+	-	N.D.	LOW
	[]	c []	+	**	N.D.	HIGH
	[]	X []	+	**	DIMER	HIGH
	X []	c []	+	+	N.D.	LOW

DEPOSITS

The following have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 in accordance with the Budapest Treaty. A plasmid clone encoding a TIE-2 ligand was deposited with the ATCC on October 7, 1994 and designated as "pJFE14 encoding TIE-2 ligand" under ATCC Accession No. 75910. Recombinant Autographa californica baculovirus encoding TIE-2 receptorbody was deposited with the ATCC on October 7, 1994 and designated as "vTIE-2 receptorbody" under ATCC Accession No. VR2484. A lambda phage vector containing human tie-2 ligand DNA was deposited with the ATCC on October 26, 1994 and designated as "lgt10 encoding htie-2 ligand 1" under ATCC Accession No. 75928. A plasmid clone encoding a second TIE-2 ligand was deposited with the ATCC on December 9, 1994 and designated as "pBluescript KS encoding human TIE 2 ligand 2" under ATCC Accession No. 75963. *E. coli* strain DH10B containing plasmid pBeLoBac11 with a human TL-4 gene insert encoding human TIE ligand-4 was deposited with the ATCC on July 2, 1996 and designated as "hTL-4" under ATCC Accession No. 98095.

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(1) APPLICANT: REGENERON PHARMACEUTICALS, INC.

(11) TITLE OF THE INVENTION: NOVEL MODIFIED LIGANDS

(111) NUMBER OF SEQUENCES: 28

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Regeneron Pharmaceuticals, Inc.
- (B) STREET: 777 Old Saw Mill Road
- (C) CITY: Tarrytown
- (D) STATE: NY
- (E) COUNTRY: USA
- (F) ZIP: 10591

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: DOS
- (D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: NOT YET KNOWN
- (B) FILING DATE: FILED HEREWITH
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: USSN 08/740,223
- (B) FILING DATE: 25-OCT-1996
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: USSN 60/022/999
- (B) FILING DATE: 02-AUG-1996

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Cobert, Robert J
- (B) REGISTRATION NUMBER: 36,108
- (C) REFERENCE/DOCKET NUMBER: REG 333

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 914-345-7400
- (B) TELEFAX: 914-345-7721

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2149 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 310...1803

(D) OTHER INFORMATION:

(A) NAME/KEY: Human TIE-2 ligand 1
 (B) LOCATION: 1...2149
 (D) OTHER INFORMATION: from clone *lgt10* encoding
htie-2 ligand 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGCTGACTC	ACGCAGGCTC	CATGCTGAAC	GGTCACACAG	AGAGGAAACA	ATAAAATCTCA	60
GCTACTATGC	AATAAATATC	TCAAGTTTA	ACGAAGAAAA	ACATCATTGC	AGTGAAATAA	120
AAAATTTAA	AATTTAGAA	CAAAGCTAAC	AAATGGCTAG	TTTTCTATGA	TTCTTCTTCA	180
AAACGCTTTCT	TTGAGGGGA	AAGAGTCAAA	CAAACAAGCA	GTTCACCTG	AAATAAAGAA	240
CTAGTTTAG	AGGTCAAGAG	AAAGGAGCAA	GTTCGAG	AGGCACGGAA	GGAGTGTGCT	300
GGCAGTACA	ATG ACA GTT	TTC CTT TCC	TTT GCT	TTC CTC GCT	GCC ATT CTG	351
Met Thr Val Phe	Leu Ser Phe	Ala Phe	Ala Ala	Ile Leu		
1	5	10				
ACT CAC ATA GGG TGC	AGC AAT CAG CGC CGA	AGT CCA GAA AAC	AGT GGG			399
Thr His Ile Gly	Cys Ser Asn Gln	Arg Arg Ser	Pro Glu Asn Ser	Gly		
15	20	25	30			
AGA AGA TAT AAC CGG ATT CAA CAT GGG CAA	TGT GCC TAC ACT	TTC ATT				447
Arg Arg Tyr Asn Arg Ile Gln His	Gly Gln Cys Ala	Tyr Thr Phe Ile				
35	40	45				
CTT CCA GAA CAC GAT GGC AAC TGT CGT	GAG AGT ACG ACA GAC CAG	TAC				495
Leu Pro Glu His Asp Gly Asn Cys Arg	Glu Ser Thr Thr Asp Gln	Tyr				
50	55	60				
AAC ACA AAC GCT CTG CAG AGA GAT GCT	CCA CAC GTG GAA CCG GAT	TTC				543
Asn Thr Asn Ala Leu Gln Arg Asp Ala	Pro His Val Glu Pro Asp Phe					
65	70	75				
TCT TCC CAG AAA CTT CAA CAT CTG GAA CAT	GTG ATG GAA AAT TAT	ACT				591
Ser Ser Gln Lys Leu Gln His Leu Glu His	Val Met Glu Asn Tyr Thr					
80	85	90				
CAG TGG CTG CAA AAA CTT GAG AAT TAC ATT	GTG GAA AAC ATG AAG TCG					639
Gln Trp Leu Gln Lys Leu Glu Asn Tyr Ile	Val Glu Asn Met Lys Ser					
95	100	105	110			
GAG ATG GCC CAG ATA CAG CAG AAT GCA GTT	CAG AAC CAC ACG GCT ACC					687
Glu Met Ala Gln Ile Gln Gln Asn Ala Val	Gln Asn His Thr Ala Thr					
115	120	125				
ATG CTG GAG ATA GGA ACC AGC CTC CTC	TCT CAG ACT GCA GAG CAG ACC					735
Met Leu Glu Ile Gly Thr Ser Leu Leu Ser	Gln Thr Ala Glu Gln Thr					
130	135	140				
AGA AAG CTG ACA GAT GTT GAG ACC CAG	GTA CTA AAT CAA ACT TCT CGA					783
Arg Lys Leu Thr Asp Val Glu Thr Gln Val	Leu Asn Gln Thr Ser Arg					
145	150	155				
CTT GAG ATA CAG CTG CTG GAG AAT TCA TTA	TCC ACC TAC AAG CTA GAG					831
Leu Glu Ile Gln Leu Leu Glu Asn Ser Leu	Ser Thr Tyr Lys Leu Glu					
160	165	170				
AAG CAA CTT CTT CAA CAG ACA AAT GAA ATC	TTG AAG ATC CAT GAA AAA					879
Lys Gln Leu Leu Gln Gln Thr Asn Glu Ile	Leu Lys Ile His Glu Lys					
175	180	185	190			
AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA	ATG GAA GGA AAA CAC AAG					927
Asn Ser Leu Leu Glu His Lys Ile Leu Glu	Met Glu Gly Lys His Lys					
195	200	205				

GAA GAG TTG GAC ACC TTA AAG GAA GAG AAA GAG AAC CTT CAA GGC TTG Glu Glu Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu 210 215 220	975
GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTG CAA AAG CAA TTA AAC Val Thr Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn 225 230 235	1023
AGA GCT ACC ACC AAC AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG Arg Ala Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu 240 245 250	1071
ATG GAC ACA GTC CAC AAC CTT GTC AAT CTT TGC ACT AAA GAA CGT GTT Met Asp Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val 255 260 265 270	1119
TTA CTA AAG GGA GGA AAA AGA GAG GAA GAG AAA CCA TTT AGA GAC TGT Leu Leu Lys Gly Gly Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys 275 280 285	1167
GCA GAT GTC TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile 290 295 300	1215
TAT ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp 305 310 315	1263
GTC AAT GGG GGA GGT TGG ACT GTC ATA CAA CAT CGT GAA GAT GGA AGT Val Asn Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser 320 325 330	1311
CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT GGA AAT Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn 335 340 345 350	1359
CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT GCC ATT ACC Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr 355 360 365	1407
AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly 370 375 380	1455
AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys 385 390 395	1503
CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys 400 405 410	1551
CAG AGC AGC CTG ATC TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT Gln Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala 415 420 425 430	1599
GAT AAT GAC AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly 435 440 445	1647
TGG TGG TTT GAT GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr 450 455 460	1695
ACT GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC Thr Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr 465 470 475	1743

TTC AAA GGG CCC AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA	1791
Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg	
480	485
CCT TTA GAT TTT TGA AAG CGCA ATGTCAGAAG CGATTATGAA AGCAACAAAG AATTC	1848
Pro Leu Asp Phe	
495	
CGGAGAAGCT GCCAGGTGAG AAACTGTTG AAAACTTCAG AAGCAAACAA TATTGTCTCC	1908
CTTCCAGCAA TAAGTGGTAG TTATGTGAAG TCACCAAGGT TCTTGACCGT GAATCTGGAG	1968
CCGTTGAGT TCACAAGAGT CTCTACTTGG GGTGACAGTG CTCACGTGGC TCGACTATAG	2028
AAAATCCAC TGACTGTCGG GCTTTAAAAA GGGAAAGAAAC TGCTGAGCTT GCTGTGCTTC	2088
AAACTACTAC TGGACCTTAT TTTGAACTA TGCTAGCCAG ATGATAAATA TGTTAATT	2148
C	2149

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 498 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Human TIE-2 ligand 1

(B) LOCATION: 1...498

(D) OTHER INFORMATION: from clone λgt10 encoding htie-2 ligand 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His			
1	5	10	15
Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg			
20	25	30	
Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro			
35	40	45	
Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr			
50	55	60	
Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser			
65	70	75	80
Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp			
85	90	95	
Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met			
100	105	110	
Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu			
115	120	125	
Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys			
130	135	140	
Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu			
145	150	155	160
Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln			
165	170	175	
Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser			
180	185	190	
Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu			
195	200	205	
Leu Asp Thr Leu Lys Glu Glu Lys Asn Leu Gln Gly Leu Val Thr			
210	215	220	
Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala			
225	230	235	240
Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp			
245	250	255	
Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu			

260	265	270
Lys Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp		
275	280	285
Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile		
290	295	300
Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn		
305	310	315
Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp		
325	330	335
Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser		
340	345	350
Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln		
355	360	365
Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg		
370	375	380
Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn		
385	390	395
Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser		
405	410	415
Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn		
420	425	430
Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp		
435	440	445
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala		
450	455	460
Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys		
465	470	475
Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu		
485	490	495
Asp Phe		

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2146 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 310...1800
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Human TIE-2 ligand 1
- (B) LOCATION: 1...2146
- (D) OTHER INFORMATION: from T98G clone

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGCTGACTC AGGCAGGCTC CATGCTAAC GGTACACACAG AGAGGAAACA ATAAATCTCA	60		
GCTACTATGC AATAAATATC TCAAGTTTA ACGAAGAAAA ACATCATGAGTGAATAA	120		
AAAATTTAA AATTTAGAA CAAAGCTAAC AAATGGCTAG TTTTCTATGA TTCTCTTCA	180		
AACGCTTCT TTGAGGGGAA AAGAGTCACAAACAGCA GTTTACCTG AAATAAAGAA	240		
CTAGTTTAG AGGTCAAGAG AAAGGAGCAA GTTTGCGAG AGGCACGGAA GGAGTGTGCT	300		
GGCAGTACA ATG ACA GTT TTC CTT TTT GCT TTC CTC GCT GGC ATT CTG			
Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu			
1	5	10	
ACT CAC ATA GGG TGC AGC AAT CAG CGC CGA AGT CCA GAA AAC AGT GGG	399		
Thr His Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly			
15	20	25	30

AGA AGA TAT AAC CGG ATT CAA CAT GGG CAA TGT GCC TAC ACT TTC ATT Arg Arg Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile 35 40 45	447
CTT CCA GAA CAC GAT GGC AAC TGT CGT GAG AGT ACG ACA GAC CAG TAC Leu Pro Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr 50 55 60	495
AAC ACA AAC GCT CTG CAG AGA GAT GCT CCA CAC GTG GAA CCG GAT TTC Asn Thr Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe 65 70 75	543
TCT TCC CAG AAA CTT CAA CAT CTG GAA CAT GTG ATG GAA AAT TAT ACT Ser Ser Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr 80 85 90	591
CAG TGG CTG CAA AAA CTT GAG AAT TAC ATT GTG GAA AAC ATG AAG TCG Gln Trp Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser 95 100 105 110	639
GAG ATG GCC CAG ATA CAG CAG AAT GCA GTT CAG AAC CAC ACG GCT ACC Glu Met Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr 115 120 125	687
ATG CTG GAG ATA GGA ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC Met Leu Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr 130 135 140	735
AGA AAG CTG ACA GAT GTT GAG ACC CAG GTC CTA AAT CAA ACT TCT CGA Arg Lys Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg 145 150 155	783
CTT GAG ATA CAG CTG CTG GAG AAT TCA TTA TCC ACC TAC AAG CTA GAG Leu Glu Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu 160 165 170	831
AAG CAA CTT CTT CAA CAG ACA AAT GAA ATC TTG AAG ATC CAT GAA AAA Lys Gln Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys 175 180 185 190	879
AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA ATG GAA GGA AAA CAC AAG Asn Ser Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys 195 200 205	927
GAA GAG TTG GAC ACC TTA AAG GAA GAG AAA GAG AAC CTT CAA GGC TTG Glu Glu Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu 210 215 220	975
GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTG GAA AAG CAA TTA AAC Val Thr Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn 225 230 235	1023
AGA GCT ACC ACC AAC AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG Arg Ala Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu 240 245 250	1071
ATG GAC ACA GTC CAC AAC CTT GTC AAT CTT TGC ACT AAA GAA GTT TTA Met Asp Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Val Leu 255 260 265 270	1119
CTA AAG GGA GGA AAA AGA GAG GAA GAG AAA CCA TTT AGA GAC TGT GCA Leu Lys Gly Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala 275 280 285	1167
GAT GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr 290 295 300	1215

ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val 305 310 315	1263
AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA AGT CTA Asn Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu 320 325 330	1311
GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT GGA AAT CCC Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro 335 340 345 350	1359
TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT GCC ATT ACC AGT Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser 355 360 365	1407
CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn 370 375 380	1455
CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln 385 390 395	1503
AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln 400 405 410	1551
AGC AGC CTG ATC TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT GAT Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp 415 420 425 430	1599
AAT GAC AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp 435 440 445	1647
TGG TTT GAT GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr 450 455 460	1695
GCG GGA CAA AAC CAT CGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC Ala Gly Gln Asn His Arg Lys Leu Asn Gly Ile Lys Trp His Tyr Phe 465 470 475	1743
AAA GGG CCC AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro 480 485 490	1791
TTA GAT TTT TGA AAGCGCA ATGTCAGAAG CGATTATGAA AGCAACAAAG AAATCCGGA Leu Asp Phe 495	1849
GAAGCTGCCA GGTGAGAAC TGTTGAAAAA CTTCAGAAGC AAACAATATT GTCTCCCTTC CACCAATAAG TGGTAGTTAT GTGAAGTCAC CAAGGTTCTT GACCGTGAAT CTGGAGCCGT TTGAGTCAC AAGAGTCTCT ACTTGGGTG ACAGTGCTCA CGTGGCTCGA CTATAGAAAA CTCCACTGAC TGTCGGGCTT TAAAAAGGG AAGAAACTGCT GAGCTTGCTG TGCTTCAAAC TACTACTGGC CCTTATTTG GAACTATGGT AGCCAGATGA TAAATATGGT TAATTTC	1909 1969 2029 2089 2146

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 497 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Human TIE-2 ligand 1
 (B) LOCATION: 1...2146
 (D) OTHER INFORMATION: from T98G clone

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
 1 5 10 15
 Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
 20 25 30
 Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
 35 40 45
 Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
 50 55 60
 Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
 65 70 75 80
 Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
 85 90 95
 Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
 100 105 110
 Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
 115 120 125
 Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
 130 135 140
 Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
 145 150 155 160
 Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
 165 170 175
 Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
 180 185 190
 Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
 195 200 205
 Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
 210 215 220
 Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
 225 230 235 240
 Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
 245 250 255
 Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Val Leu Leu Lys
 260 265 270
 Gly Gly Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val
 275 280 285
 Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn
 290 295 300
 Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly
 305 310 315 320
 Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe
 325 330 335
 Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly
 340 345 350
 Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg
 355 360 365
 Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala
 370 375 380
 Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr
 385 390 395 400
 Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser
 405 410 415
 Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp
 420 425 430
 Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe
 435 440 445
 Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly

450	455	460													
Gln	Asn	His	Arg	Lys	Leu	Asn	Gly	Ile	Lys	Trp	His	Tyr	Phe	Lys	Gly
465															
470															480
Pro	Ser	Tyr	Ser	Leu	Arg	Ser	Thr	Thr	Met	Met	Ile	Arg	Pro	Leu	Asp
															495
Phe															

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2282 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 357...1844
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Human TIE-2 ligand 2

- (B) LOCATION: 1...2282

- (D) OTHER INFORMATION: from clone pBluescript KS
encoding human TIE 2 ligand 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCTGG GTTGGTGT TT ATCTCCTCCC ACCCTTGAGG GAGGGAACAA CACTGTAGGA TCTGGGGAGA GAGGAACAAA GGACCGTGAA AGCTGCTCTG TAAAAGCTGA CACAGCCCTC CCAAGTGAGC AGGACTGTTT TTCCCCTGC AATCTGACAG TTTACTGCAT GCCTGGAGAG AACACAGCAG TAAAAACACAG GTTTGCTACT GGAAAAAGAG GAAAGAGAAG ACTTCATTG ACGGACCCAG CCATGGCAGC GTAGCAGCCC TCGCTTTCAG ACGGCAGCAG CTCGGGACTC TGGACGTGTG TTTGCCCTCA AGTTTGCTAA GCTGCTGGTT TATTACTGAA GAAAGA ATG Met 1	60 120 180 240 300 359
TGG CAG ATT GTT TTC TTT ACT CTG AGC TGT GAT CTT GTC TTG GCC GCA Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala Ala 5 10 15	407
GCC TAT AAC AAC TTT CCG AAG ACC ATG GAC AGC ATA GGA AAG AAG CAA Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys Gln 20 25 30	455
TAT CAG GTC CAG CAT GGG TCC TGC AGC TAC ACT TTC CTC CTG CCA GAG Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro Glu 35 40 45	503
ATG GAC AAC TGC CGC TCT TCC TCC AGC CCC TAC GTG TCC AAT GCT GTG Met Asp Asn Cys Arg Ser Ser Ser Pro Tyr Val Ser Asn Ala Val 50 55 60 65	551
CAG AGG GAC GCG CCG CTC GAA TAC GAT GAC TCG GTG CAG AGG CTG CAA Gln Arg Asp Ala Pro Leu Glu Tyr Asp Ser Val Gln Arg Leu Gln 70 75 80	599
GTG CTG GAG AAC ATC ATG GAA AAC ACT CAG TGG CTA ATG AAG CTT Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys Leu 85 90 95	647
GAG AAT TAT ATC CAG GAC AAC ATG AAG AAA GAA ATG GTA GAG ATA CAG Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile Gln 100 105 110	695

CAG AAT GCA GTA CAG AAC CAG ACG GCT GTG ATG ATA GAA ATA GGG ACA Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly Thr 115 120 125	743
AAC CTG TTG AAC CAA ACA GCT GAG CAA ACG CGG AAG TTA ACT GAT GTG Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val 130 135 140 145	791
GAA GCC CAA GTA TTA AAT CAG ACC ACG AGA CTT GAA CTT CAG CTC TTG Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu Leu 150 155 160	839
GAA CAC TCC CTC TCG ACA AAC AAA TTG GAA AAA CAG ATT TTG GAC CAG Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp Gln 165 170 175	887
ACC AGT GAA ATA AAC AAA TTG CAA GAT AAG AAC AGT TTC CTA GAA AAG Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu Lys 180 185 190	935
AAG GTG CTA GCT ATG GAA GAC AAG CAC ATC ATC CAA CTA CAG TCA ATA Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser Ile 195 200 205	983
AAA GAA GAG AAA GAT CAG CTA CAG GTG TTA GTA TCC AAG CAA AAT TCC Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn Ser 210 215 220 225	1031
ATC ATT GAA GAA CTA GAA AAA AAA ATA GTG ACT GCC ACG GTG AAT AAT Ile Ile Glu Glu Leu Glu Lys Ile Val Thr Ala Thr Val Asn Asn 230 235 240	1079
TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC ATG GAG ACA GTT AAT AAC Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn Asn 245 250 255	1127
TTA CTG ACT ATG ATG TCC ACA TCA AAC TCA GCT AAG GAC CCC ACT GTT Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr Val 260 265 270	1175
GCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCT GAA GTA TTC AAA Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe Lys 275 280 285	1223
TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA ACA TTC CCT AAT TCT Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn Ser 290 295 300 305	1271
ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly Gly 310 315 320	1319
TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg 325 330 335	1367
ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr 340 345 350	1415
TGG CTG GGA AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr 355 360 365	1463
GTG CTT AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser 370 375 380 385	1511

TTG TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile 390 395 400	1559
CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile Ser 405 410 415	1607
CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys Cys 420 425 430	1655
ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT GCA Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp Ala 435 440 445	1703
TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln Asn 450 455 460 465	1751
ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG AAA GGC TCA GGC Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser Gly 470 475 480	1799
TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA GAT TTC TAAAC Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe 485 490 495	1849
ATCCCAGTCC ACCTGAGGAA CTGTCTCGAA CTATTTCAA AGACTTAAGC CCGTGCAC GAAAGTCACG GCTGCCACT GTGTCTCTT CCACACAGA GGGCGTGTGC TCGGTGCTGA CGGGACCCAC ATGCTCCAGA TTAGAGCCTG TAAACTTTAT CACTTAAACT TGCATCACTT AACGGACCA AGCAAGACCC TAAACATCCA TAATTGTGAT TAGACAGAAC ACCTATGCAA AGATGAACCC GAGGCTGAGA ATCAGACTGA CAGTTTACAG ACGCTGCTGT CACAACCAAG AATGTTATGT GCAAGTTAT CAGTAAATAA CTGAAAACA GAACACTTAT GTTATACAAT ACAGATCATC TTGGAACCTGC ATTCTTCTGA GCACGTGTTA TACACTGTGT AAATACCCAT ATGCTCTGAA TTC	1909 1969 2029 2089 2149 2209 2269 2282

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 496 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (v) FRAGMENT TYPE: internal
- (ix) FEATURE:

- (A) NAME/KEY: Human TIE-2 ligand 2

- (B) LOCATION: 1...496

- (D) OTHER INFORMATION: from clone pBluescript KS
encoding human TIE 2 ligand 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala 1 5 10 15
Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys 20 25 30
Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro 35 40 45
Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala 50 55 60
Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu 65 70 75 80
Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys

85	90	95
Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile		
100	105	110
Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly		
115	120	125
Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp		
130	135	140
Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu		
145	150	155
Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp		
165	170	175
Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu		
180	185	190
Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser		
195	200	205
Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn		
210	215	220
Ser Ile Ile Glu Glu Leu Glu Lys Ile Val Thr Ala Thr Val Asn		
225	230	235
Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn		
245	250	255
Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr		
260	265	270
Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe		
275	280	285
Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn		
290	295	300
Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly		
305	310	315
Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln		
325	330	335
Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu		
340	345	350
Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg		
355	360	365
Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr		
370	375	380
Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg		
385	390	395
Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile		
405	410	415
Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys		
420	425	430
Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp		
435	440	445
Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln		
450	455	460
Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser		
465	470	475
Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe		
485	490	495

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 478 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Mature TL1 protein

- (B) LOCATION: 1...478

- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg Tyr Asn Arg Ile
 1 5 10 15
 Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro Glu His Asp Gly
 20 25 30
 Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr Asn Ala Leu Gln
 35 40 45
 Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser Gln Lys Leu Gln
 50 55 60
 His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys Leu
 65 70 75 80
 Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile Gln
 85 90 95
 Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly Thr
 100 105 110
 Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val
 115 120 125
 Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu Leu
 130 135 140
 Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln Gln
 145 150 155 160
 Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu His
 165 170 175
 Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Leu Asp Thr Leu
 180 185 190
 Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr Tyr
 195 200 205
 Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn Asn
 210 215 220
 Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His Asn
 225 230 235 240
 Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu Lys Gly Lys Lys
 245 250 255
 Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln Ala
 260 265 270
 Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro
 275 280 285
 Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp
 290 295 300
 Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly
 305 310 315 320
 Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp
 325 330 335
 Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met
 340 345 350
 Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln
 355 360 365
 Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr
 370 375 380
 Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu
 385 390 395 400
 His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met
 405 410 415
 Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys
 420 425 430
 Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His
 435 440 445
 Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser Tyr
 450 455 460
 Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
 465 470 475

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 480 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (ix) FEATURE:

(A) NAME/KEY: Mature TL2 protein
 (B) LOCATION: 1...480
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
 1 5 10 15
 Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
 20 25 30
 Glu Met Asp Asn Cys Arg Ser Ser Ser Pro Tyr Val Ser Asn Ala
 35 40 45
 Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu
 50 55 60
 Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys
 65 70 75 80
 Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile
 85 90 95
 Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
 100 105 110
 Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
 115 120 125
 Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
 130 135 140
 Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
 145 150 155 160
 Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
 165 170 175
 Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
 180 185 190
 Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
 195 200 205
 Ser Ile Ile Glu Glu Leu Glu Lys Ile Val Thr Ala Thr Val Asn
 210 215 220
 Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn
 225 230 235 240
 Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
 245 250 255
 Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe
 260 265 270
 Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn
 275 280 285
 Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly Gly
 290 295 300
 Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln
 305 310 315 320
 Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu
 325 330 335
 Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg
 340 345 350
 Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr
 355 360 365
 Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg
 370 375 380
 Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile
 385 390 395 400
 Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys
 405 410 415

Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp
 420 425 430
 Ala Cys Gly Pr Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln
 435 440 445
 Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser
 450 455 460
 Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
 465 470 475 480

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1849 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 47...1573
- (D) OTHER INFORMATION:

- (A) NAME/KEY: TIE ligand-3
- (B) LOCATION: 1...1849
- (D) OTHER INFORMATION: The fibrinogen-like domain starts at position 929.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGTCCCTGGT ACCTGACAAG ACCACCTCAC CACCACTTGG TCTCAG ATG CTC TGC	55
Met Leu Cys	
1	
CAG CCA GCT ATG CTA CTA GAT GGC CTC CTC CTG CTG GCC ACC ATG GCT	103
Gln Pro Ala Met Leu Leu Asp Gly Leu Leu Leu Ala Thr Met Ala	
5 10 15	
GCA GCC CAG CAC AGA GGG CCA GAA GCC GGT GGG CAC CGC CAG ATT CAC	151
Ala Ala Gln His Arg Gly Pro Glu Ala Gly Gly His Arg Gln Ile His	
20 25 30 35	
CAG GTC CGG CGT GGC CAG TGC AGC TAC ACC TTT GTG GTG CCG GAG CCT	199
Gln Val Arg Arg Gly Gln Cys Ser Tyr Thr Phe Val Val Pro Glu Pro	
40 45 50	
GAT ATC TGC CAG CTG GCG CCG ACA GCG GCG CCT GAG GCT TTG GGG GGC	247
Asp Ile Cys Gln Leu Ala Pro Thr Ala Ala Pro Glu Ala Leu Gly Gly	
55 60 65	
TCC AAT AGC CTC CAG AGG GAC TTG CCT GCC TCG AGG CTG CAC CTA ACA	295
Ser Asn Ser Leu Gln Arg Asp Leu Pro Ala Ser Arg Leu His Leu Thr	
70 75 80	
GAC TGG CGA GCC CAG AGG GCC CAG CGG GCC CAG CGT GTG AGC CAG CTG	343
Asp Trp Arg Ala Gln Arg Ala Gln Arg Ala Gln Arg Val Ser Gln Leu	
85 90 95	
GAG AAG ATA CTA GAG AAT AAC ACT CAG TGG CTG CTG AAG CTG GAG CAG	391
Glu Lys Ile Leu Glu Asn Asn Thr Gln Trp Leu Leu Lys Leu Glu Gln	
100 105 110 115	
TCC ATC AAG GTG AAC TTG AGG TCA CAC CTG GTG CAG GCC CAG CAG GAC	439
Ser Ile Lys Val Asn Leu Arg Ser His Leu Val Gln Ala Gln Gln Asp	
120 125 130	

ACA ATC CAG AAC CAG ACA ACT ACC ATG CTG GCA CTG GGT GCC AAC CTC Thr Ile Gln Asn Gln Thr Thr Thr Met Leu Ala Leu Gly Ala Asn Leu 135 140 145	487
ATG AAC CAG ACC AAA GCT CAG ACC CAC AAG CTG ACT GCT GTG GAG GCA Met Asn Gln Thr Lys Ala Gln Thr His Lys Leu Thr Ala Val Glu Ala 150 155 160	535
CAG GTC CTA AAC CAG ACA TTG CAC ATG AAG ACC CAA ATG CTG GAG AAC Gln Val Leu Asn Gln Thr Leu His Met Lys Thr Gln Met Leu Glu Asn 165 170 175	583
TCA CTG TCC ACC AAC AAG CTG GAG CGG CAG ATG CTG ATG CAG AGC CGA Ser Leu Ser Thr Asn Lys Leu Glu Arg Gln Met Leu Met Gln Ser Arg 180 185 190 195	631
GAG CTG CAG CGG CTG CAG GGT CGC AAC AGG GCC CTG GAG ACC AGG CTG Glu Leu Gln Arg Leu Gln Gly Arg Asn Arg Ala Leu Glu Thr Arg Leu 200 205 210	679
CAG GCA CTG GAA GCA CAA CAT CAG GCC CAG CTT AAC AGC CTC CAA GAG Gln Ala Leu Glu Ala Gln His Gln Ala Gln Leu Asn Ser Leu Gln Glu 215 220 225	727
AAG AGG GAA CAA CTG CAC AGT CTC CTG GGC CAT CAG ACC GGG ACC CTG Lys Arg Glu Gln Leu His Ser Leu Leu Gly His Gln Thr Gly Thr Leu 230 235 240	775
GCT AAC CTG AAG CAC AAT CTG CAC GCT CTC AGC AGC AAT TCC AGC TCC Ala Asn Leu Lys His Asn Leu His Ala Leu Ser Ser Asn Ser Ser Ser 245 250 255	823
CTG CAG CAG CAG CAG CAA CTG ACG GAG TTT GTA CAG CGC CTG GTA Leu Gln Gln Gln Gln Leu Thr Glu Phe Val Gln Arg Leu Val 260 265 270 275	871
CGG ATT GTA GCC CAG GAC CAG CAT CCG GTT TCC TTA AAG ACA CCT AAG Arg Ile Val Ala Gln Asp Gln His Pro Val Ser Leu Lys Thr Pro Lys 280 285 290	919
CCA GTG TTC CAG GAC TGT GCA GAG ATC AAG CGC TCC GGG GTT AAT ACC Pro Val Phe Gln Asp Cys Ala Glu Ile Lys Arg Ser Gly Val Asn Thr 295 300 305	967
AGC GGT GTC TAT ACC ATC TAT GAG ACC AAC ATG ACA AAG CCT CTC AAG Ser Gly Val Tyr Thr Ile Tyr Glu Thr Asn Met Thr Lys Pro Leu Lys 310 315 320	1015
GTG TTC TGT GAC ATG GAG ACT GAT GGA GGT GCC TGG ACC CTC ATC CAG Val Phe Cys Asp Met Glu Thr Asp Gly Gly Gly Trp Thr Leu Ile Gln 325 330 335	1063
CAC CGG GAG GAT GGA AGC GTA AAT TTC CAG AGG ACC TGG GAA GAA TAC His Arg Glu Asp Gly Ser Val Asn Phe Gln Arg Thr Trp Glu Glu Tyr 340 345 350 355	1111
AAA GAG GGT TTT GGT AAT GTG GCC AGA GAG CAC TGG CTG GGC AAT GAG Lys Glu Gly Phe Gly Asn Val Ala Arg Glu His Trp Leu Gly Asn Glu 360 365 370	1159
GCT GTG CAC CGC CTC ACC AGC AGA ACG GCC TAC TTG CTA CGC GTG GAA Ala Val His Arg Leu Thr Ser Arg Thr Ala Tyr Leu Leu Arg Val Glu 375 380 385	1207
CTG CAT GAC TGG GAA GGC CGC CAG ACC TCC ATC CAG TAT GAG AAC TTC Leu His Asp Trp Glu Gly Arg Gln Thr Ser Ile Gln Tyr Glu Asn Phe 390 395 400	1255

CAG CTG GGC AGC GAG AGG CAG CGG TAC AGC CTC TCT GTG AAT GAC AGC Gln Leu Gly Ser Glu Arg Gln Arg Tyr Ser Leu Ser Val Asn Asp Ser 405 410 415	1303
AGC AGT TCA GCA GGG CGC AAG AAC AGC CTG GCT CCT CAG GGC ACC AAG Ser Ser Ser Ala Gly Arg Lys Asn Ser Leu Ala Pro Gln Gly Thr Lys 420 425 430 435	1351
TTC AGC ACC AAA GAC ATG GAC AAT GAT AAC TGC ATG TGT AAA TGT GCT Phe Ser Thr Lys Asp Met Asp Asn Asp Asn Cys Met Cys Lys Cys Ala 440 445 450	1399
CAG ATG CTG TCT GGA GGG TGG TGG TTT GAT GCC TGT GGC CTC TCC AAC Gln Met Leu Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn 455 460 465	1447
CTC AAT GGC ATC TAC TAT TCA GTT CAT CAG CAC TTG CAC AAG ATC AAT Leu Asn Gly Ile Tyr Tyr Ser Val His Gln His Leu His Lys Ile Asn 470 475 480	1495
GGC ATC CGC TGG CAC TAC TTC CGA GGC CCC AGC TAC TCA CTG CAC GGC Gly Ile Arg Trp His Tyr Phe Arg Gly Pro Ser Tyr Ser Leu His Gly 485 490 495	1543
ACA CGC ATG ATG CTG AGG CCA ATG GGT GCC TGA CACACAG CCCTGCAGAG ACT Thr Arg Met Met Leu Arg Pro Met Gly Ala 500 505	1596
GATGCCGTAG GAGGATTCTC AACCCAGGTG ACTCTGTGCA CGCTGGGCC TGCCCGAGAAA TCAGTCCCCA GGGCTCATCT TGACATTCTG GAACATCGGA ACCAGCTTAC CTTGCCCCCTG AATTACAAGA ATTACACCTGC CTCCCTGTTG CCCTCTAATT GTGAAATTGC TGGGTGCTTG AAGGCACCTG CCTCTGTTGG AACCATACTC TTTCCTCTC CTGCTGCATG CCCGGGAATC CCTGCCATGA ACT	1656 1716 1776 1836 1849

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 509 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (v) FRAGMENT TYPE: internal
- (ix) FEATURE:

- (A) NAME/KEY: TIE ligand-3
- (B) LOCATION: 1...509
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Leu Cys Gln Pro Ala Met Leu Leu Asp Gly Leu Leu Leu Ala
1 5 10 15
Thr Met Ala Ala Ala Gln His Arg Gly Pro Glu Ala Gly Gly His Arg
20 25 30
Gln Ile His Gln Val Arg Arg Gly Gln Cys Ser Tyr Thr Phe Val Val
35 40 45
Pro Glu Pro Asp Ile Cys Gln Leu Ala Pro Thr Ala Ala Pro Glu Ala
50 55 60
Leu Gly Gly Ser Asn Ser Leu Gln Arg Asp Leu Pro Ala Ser Arg Leu
65 70 75 80
His Leu Thr Asp Trp Arg Ala Gln Arg Ala Gln Arg Ala Gln Arg Val
85 90 95
Ser Gln Leu Glu Lys Ile Leu Glu Asn Asn Thr Gln Trp Leu Leu Lys

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100	105	110
Leu Glu Gln Ser Ile Lys Val Asn Leu Arg Ser His	Leu Val Gln Ala	
115	120	125
Gln Gln Asp Thr Ile Gln Asn Gln Thr Thr Met	Leu Ala Leu Gly	
130	135	140
Ala Asn Leu Met Asn Gln Thr Lys Ala Gln Thr His	Lys Leu Thr Ala	
145	150	155
Val Glu Ala Gln Val Leu Asn Gln Thr Leu His Met	Lys Thr Gln Met	160
165	170	175
Leu Glu Asn Ser Leu Ser Thr Asn Lys Leu Glu Arg	Gln Met Leu Met	
180	185	190
Gln Ser Arg Glu Leu Gln Arg Leu Gln Gly Arg Asn	Arg Ala Leu Glu	
195	200	205
Thr Arg Leu Gln Ala Leu Glu Ala Gln His Gln Ala	Gln Leu Asn Ser	
210	215	220
Leu Gln Glu Lys Arg Glu Gln Leu His Ser Leu Leu Gly	His Gln Thr	
225	230	235
Gly Thr Leu Ala Asn Leu Lys His Asn Leu His Ala Leu	Ser Ser Asn	240
245	250	255
Ser Ser Ser Leu Gln Gln Gln Gln Gln Leu Thr Glu Phe	Val Gln	
260	265	270
Arg Leu Val Arg Ile Val Ala Gln Asp Gln His Pro	Val Ser Leu Lys	
275	280	285
Thr Pro Lys Pro Val Phe Gln Asp Cys Ala Glu Ile	Lys Arg Ser Gly	
290	295	300
Val Asn Thr Ser Gly Val Tyr Thr Ile Tyr Glu Thr Asn	Met Thr Lys	
305	310	315
Pro Leu Lys Val Phe Cys Asp Met Glu Thr Asp Gly Gly	Gly Trp Thr	320
325	330	335
Leu Ile Gln His Arg Glu Asp Gly Ser Val Asn Phe Gln	Arg Thr Trp	
340	345	350
Glu Glu Tyr Lys Glu Gly Phe Gly Asn Val Ala Arg	Glu His Trp Leu	
355	360	365
Gly Asn Glu Ala Val His Arg Leu Thr Ser Arg Thr	Ala Tyr Leu Leu	
370	375	380
Arg Val Glu Leu His Asp Trp Glu Gly Arg Gln Thr	Ser Ile Gln Tyr	
385	390	395
Glu Asn Phe Gln Leu Gly Ser Glu Arg Gln Arg Tyr	Ser Leu Ser Val	400
405	410	415
Asn Asp Ser Ser Ser Ala Gly Arg Lys Asn Ser Leu Ala	Pro Gln	
420	425	430
Gly Thr Lys Phe Ser Thr Lys Asp Met Asp Asn Asn	Cys Met Cys	
435	440	445
Lys Cys Ala Gln Met Leu Ser Gly Gly Trp Trp Phe	Asp Ala Cys Gly	
450	455	460
Leu Ser Asn Leu Asn Gly Ile Tyr Tyr Ser Val His	Gln His Leu His	
465	470	475
Lys Ile Asn Gly Ile Arg Trp His Tyr Phe Arg Gly	Pro Ser Tyr Ser	480
485	490	495
Leu His Gly Thr Arg Met Met Leu Arg Pro Met Gly	Ala	
500	505	

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 503 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: mTL3
- (B) LOCATION: 1...503
- (D) OTHER INFORMATION: mouse TIE ligand-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Leu Leu Asp Gly Leu Leu Leu Leu Ala Thr Met Ala Ala Ala Gln
 1 5 10 15
 His Arg Gly Pro Glu Ala Gly Gly His Arg Gln Ile His Gln Val Arg
 20 25 30
 Arg Gly Gln Cys Ser Tyr Thr Phe Val Val Pro Glu Pro Asp Ile Cys
 35 40 45
 Gln Leu Ala Pro Thr Ala Ala Pro Glu Ala Leu Gly Gly Ser Asn Ser
 50 55 60
 Leu Gln Arg Asp Leu Pro Ala Ser Arg Leu His Leu Thr Asp Trp Arg
 65 70 75 80
 Ala Gln Arg Ala Gln Arg Ala Gln Arg Val Ser Gln Leu Glu Lys Ile
 85 90 95
 Leu Glu Asn Asn Thr Gln Trp Leu Leu Lys Leu Glu Gln Ser Ile Lys
 100 105 110
 Val Asn Leu Arg Ser His Leu Val Gln Ala Gln Gln Asp Thr Ile Gln
 115 120 125
 Asn Gln Thr Thr Thr Met Leu Ala Leu Gly Ala Asn Leu Met Asn Gln
 130 135 140
 Thr Lys Ala Gln Thr His Lys Leu Thr Ala Val Glu Ala Gln Val Leu
 145 150 155 160
 Asn Gln Thr Leu His Met Lys Thr Gln Met Leu Glu Asn Ser Leu Ser
 165 170 175
 Thr Asn Lys Leu Glu Arg Gln Met Leu Met Gln Ser Arg Glu Leu Gln
 180 185 190
 Arg Leu Gln Gly Arg Asn Arg Ala Leu Glu Thr Arg Leu Gln Ala Leu
 195 200 205
 Glu Ala Gln His Gln Ala Gln Leu Asn Ser Leu Gln Glu Lys Arg Glu
 210 215 220
 Gln Leu His Ser Leu Leu Gly His Gln Thr Gly Thr Leu Ala Asn Leu
 225 230 235 240
 Lys His Asn Leu His Ala Leu Ser Ser Asn Ser Ser Ser Leu Gln Gln
 245 250 255
 Gln Gln Gln Leu Thr Glu Phe Val Gln Arg Leu Val Arg Ile Val
 260 265 270
 Ala Gln Asp Gln His Pro Val Ser Leu Lys Thr Pro Lys Pro Val Phe
 275 280 285
 Gln Asp Cys Ala Glu Ile Lys Arg Ser Gly Val Asn Thr Ser Gly Val
 290 295 300
 Tyr Thr Ile Tyr Glu Thr Asn Met Thr Lys Pro Leu Lys Val Phe Cys
 305 310 315 320
 Asp Met Glu Thr Asp Gly Gly Trp Thr Leu Ile Gln His Arg Glu
 325 330 335
 Asp Gly Ser Val Asn Phe Gln Arg Thr Trp Glu Glu Tyr Lys Glu Gly
 340 345 350
 Phe Gly Asn Val Ala Arg Glu His Trp Leu Gly Asn Glu Ala Val His
 355 360 365
 Arg Leu Thr Ser Arg Thr Ala Tyr Leu Leu Arg Val Glu Leu His Asp
 370 375 380
 Trp Glu Gly Arg Gln Thr Ser Ile Gln Tyr Glu Asn Phe Gln Leu Gly
 385 390 395 400
 Ser Glu Arg Gln Arg Tyr Ser Leu Ser Val Asn Asp Ser Ser Ser Ser
 405 410 415
 Ala Gly Arg Lys Asn Ser Leu Ala Pro Gln Gly Thr Lys Phe Ser Thr
 420 425 430
 Lys Asp Met Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Gln Met Leu
 435 440 445
 Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn Leu Asn Gly
 450 455 460
 Ile Tyr Tyr Ser Val His Gln His Leu His Lys Ile Asn Gly Ile Arg
 465 470 475 480
 Trp His Tyr Phe Arg Gly Pro Ser Tyr Ser Ile His Gly Thr Arg Met
 485 490 495
 Met Leu Arg Pro Met Gly Ala

500

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 490 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: htl1
- (B) LOCATION: 1...490
- (D) OTHER INFORMATION: human TIE-2 ligand 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Phe Leu Ala Ala Ile Leu Thr His Ile Gly Cys Ser Asn Gln Arg
 1 5 10 15
 Arg Ser Pro Glu Asn Ser Gly Arg Arg Tyr Asn Arg Ile Gln His Gly
 20 25 30
 Gln Cys Ala Tyr Thr Phe Ile Leu Pro Glu His Asp Gly Asn Cys Arg
 35 40 45
 Glu Ser Thr Thr Asp Gln Tyr Asn Thr Asn Ala Leu Gln Arg Asp Ala
 50 55 60
 Pro His Val Glu Pro Asp Phe Ser Ser Gln Lys Leu Gln His Leu Glu
 65 70 75 80
 His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys Leu Glu Asn Tyr
 85 90 95
 Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile Gln Gln Asn Ala
 100 105 110
 Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly Thr Ser Leu Leu
 115 120 125
 Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val Glu Thr Gln
 130 135 140
 Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu Leu Glu Asn Ser
 145 150 155 160
 Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln Gln Thr Asn Glu
 165 170 175
 Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu His Lys Ile Leu
 180 185 190
 Glu Met Glu Gly Lys His Lys Glu Leu Asp Thr Leu Lys Glu Glu
 195 200 205
 Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr Tyr Ile Ile Gln
 210 215 220
 Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn Asn Ser Val Leu
 225 230 235 240
 Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His Asn Leu Val Asn
 245 250 255
 Leu Cys Thr Lys Glu Val Leu Leu Lys Gly Gly Lys Arg Glu Glu Glu
 260 265 270
 Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys
 275 280 285
 Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys
 290 295 300
 Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln
 305 310 315 320
 His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr
 325 330 335
 Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu
 340 345 350
 Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu
 355 360 365
 Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe

370	375	380	
His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys		ly His	
385	390	395	400
Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp			
405	410	415	
Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys Ala			
420	425	430	
Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn			
435	440	445	
Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu Asn			
450	455	460	
Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser Tyr Ser Ile Arg Ser			
465	470	475	480
Thr Thr Met Met Ile Arg Pro Leu Asp Phe			
485	490		

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 491 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: chTL1
- (B) LOCATION: 1...491
- (D) OTHER INFORMATION: chicken TIE-2 ligand 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Phe Leu Ala Ala Ile Leu Ala His Ile Gly Cys Thr Thr Gln Arg			
1	5	10	15
Arg Ser Pro Glu Asn Ser Gly Arg Arg Phe Asn Arg Ile Gln His Gly			
20	25	30	
Gln Cys Thr Tyr Thr Phe Ile Leu Pro Glu Gln Asp Gly Asn Cys Arg			
35	40	45	
Glu Ser Thr Thr Asp Gln Tyr Asn Thr Asn Ala Leu Gln Arg Asp Ala			
50	55	60	
Pro His Val Glu Gln Asp Phe Ser Phe Gln Lys Leu Gln His Leu Glu			
65	70	75	80
His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys Leu Glu Ser Tyr			
85	90	95	
Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Leu Gln Asn Ala			
100	105	110	
Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly Thr Ser Leu Leu			
115	120	125	
Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val Glu Thr Gln			
130	135	140	
Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu Leu Glu Asn Ser			
145	150	155	160
Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln Gln Thr Asn Glu			
165	170	175	
Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu His Lys Ile Leu			
180	185	190	
Glu Met Glu Glu Arg His Lys Glu Glu Met Asp Thr Leu Lys Glu Glu			
195	200	205	
Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Ser Tyr Ile Ile Gln			
210	215	220	
Glu Leu Glu Lys Gln Leu Asn Lys Ala Thr Thr Asn Asn Ser Val Leu			
225	230	235	240
Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His Thr Leu Ile Thr			
245	250	255	
Leu Cys Ser Lys Glu Gly Val Leu Leu Lys Asn Ala Lys Arg Glu Glu			

260	265	270
Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn		
275	280	285
Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Val Ser Asp Pro Lys		
290	295	300
Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile		
305	310	315
Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Lys Gly Trp Lys Glu		
320	325	330
Tyr Lys Met Gly Phe Gly Ser Pro Ser Gly Glu Tyr Trp Leu Gly Asn		
340	345	350
Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Ser Leu Arg Ile		
355	360	365
Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg		
370	375	380
Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly		
385	390	395
His Ser Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala		
405	410	415
Glu Phe Ser Thr Lys Asp Ala Asp Asn Asn Cys Met Cys Lys Cys		
420	425	430
Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser		
435	440	445
Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu		
450	455	460
Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Arg Tyr Ser Ile Arg		
465	470	475
Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe		
485	490	

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 497 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: mTL1
- (B) LOCATION: 1...497
- (D) OTHER INFORMATION: mouse TIE-2 ligand 1

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Thr Val Phe Leu Ser Phe Ala Phe Phe Ala Ala Ile Leu Thr His			
1	5	10	15
Ile Gly Cys Ser Asn Gln Arg Arg Asn Pro Glu Asn Ser Gly Arg Arg			
20	25	30	
Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro			
35	40	45	
Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr			
50	55	60	
Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser			
65	70	75	80
Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp			
85	90	95	
Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met			
100	105	110	
Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu			
115	120	125	
Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys			
130	135	140	
Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu			

145	150	155	160
Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln			
165	170	175	
Leu Leu Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu			
180	185	190	
Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Met			
195	200	205	
Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Ser Arg			
210	215	220	
Gln Ser Phe Ile Ile Gln Glu Leu Glu Lys Gln Leu Ser Arg Ala Thr			
225	230	235	240
Asn Asn Asn Ser Ile Leu Gln Lys Gln Gln Leu Glu Leu Met Asp Thr			
245	250	255	
Val His Asn Leu Ile Ser Leu Cys Thr Lys Glu Gly Val Leu Leu Lys			
260	265	270	
Gly Gly Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val			
275	280	285	
Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Phe Asn			
290	295	300	
Asn Val Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly			
305	310	315	320
Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe			
325	330	335	
Gln Lys Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Ser Pro Ser Gly			
340	345	350	
Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg			
355	360	365	
Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala			
370	375	380	
Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr			
385	390	395	400
Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser			
405	410	415	
Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp			
420	425	430	
Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe			
435	440	445	
Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly			
450	455	460	
Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly			
465	470	475	480
Pro Arg Tyr Ser Ile Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp			
485	490	495	
Phe			

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 496 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: mTL2
- (B) LOCATION: 1...496
- (D) OTHER INFORMATION: mouse TIE-2 ligand 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Trp Gln Ile Ile Phe Leu Thr Phe Gly Trp Asp Ala Val Leu Thr			
1	5	10	15
Ser Ala Tyr Ser Asn Phe Arg Lys Ser Val Asp Ser Thr Gly Arg Arg			

20	25	30
Arg Tyr Arg Ile Gln Asn Gly Pro Cys Ala Tyr Thr Phe Leu Leu Pro		
35	40	45
Glu Thr Asp Ser Gly Arg Ser Ser Ser Thr Tyr Met Thr Asn Ala		
50	55	60
Val Gln Arg Asp Ala Pro Pro Asp Tyr Glu Asp Ser Val Gln Ser Leu		
65	70	75
Gln Leu Leu Glu Asn Val Met Glu Asn Tyr Thr Gln Trp Leu Met Lys		
85	90	95
Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Ala Glu Ile		
100	105	110
Gln Gln Asn Val Val Gln Asn His Thr Ala Val Met Ile Glu Ile Gly		
115	120	125
Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp		
130	135	140
Val Glu Thr Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu		
145	150	155
Leu Gln His Ser Ile Ser Thr Tyr Lys Leu Glu Lys Gln Ile Leu Asp		
165	170	175
Gln Thr Ser Glu Ile Asn Lys Ile His Asn Lys Asn Ser Phe Leu Glu		
180	185	190
Gln Lys Val Leu Asp Met Glu Gly Lys His Ser Glu Glu Met Gln Thr		
195	200	205
Met Lys Glu Gln Lys Asp Glu Leu Gln Val Leu Val Ser Lys Gln Ser		
210	215	220
Ser Val Ile Asp Glu Leu Glu Lys Lys Leu Val Thr Ala Thr Val Asn		
225	230	235
Asn Ser Leu Leu Gln Lys Gln His Asp Leu Met Asp Thr Val Asn		
245	250	255
Ser Leu Leu Thr Met Met Ser Ser Pro Asn Ser Lys Ser Ser Leu Ala		
260	265	270
Ile Arg Arg Glu Glu Gln Thr Thr Phe Arg Asp Cys Ala Asp Val Phe		
275	280	285
Lys Ala Gly Leu Thr Lys Ser Gly Ile Tyr Thr Leu Thr Phe Pro Asn		
290	295	300
Ser Pro Glu Glu Ile Lys Ala Tyr Cys Asn Met Asp Val Gly Gly		
305	310	315
Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln		
325	330	335
Lys Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Leu Gly Glu		
340	345	350
Tyr Trp Leu Gly Asn Glu Phe Ile Ser Gln Ile Thr Gly Gln His Arg		
355	360	365
Tyr Val Leu Lys Ile Gln Leu Lys Asp Trp Glu Gly Asn Glu Ala His		
370	375	380
Ser Leu Tyr Asp His Phe Tyr Ile Ala Gly Glu Glu Ser Asn Tyr Arg		
385	390	395
Ile His Leu Thr Gly Leu Thr Gly Thr Ala Ala Lys Ile Ser Ser Ile		
405	410	415
Ser Gln Pro Gly Ser Asp Phe Ser Thr Lys Asp Ser Asp Asn Asp Lys		
420	425	430
Cys Ile Cys Lys Cys Ser Leu Met Leu Thr Gly Gly Trp Trp Phe Asp		
435	440	445
Ala Cys Gly Pro Ser Asn Leu Asn Gln Phe Tyr Pro Gln Lys Gln		
450	455	460
Asn Thr Asn Lys Phe Asn Gln Ile Lys Trp Tyr Tyr Trp Lys Gly Ser		
465	470	475
Gly Tyr Ser Ile Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe		
485	490	495

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 496 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (ix) FEATURE:

(A) NAME/KEY: hTL2
 (B) LOCATION: 1...496
 (D) OTHER INFORMATION: human TIE-2 ligand 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Ala Val Leu Thr
 1 5 10 15
 Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
 20 25 30
 Arg Tyr Arg Ile Gln His Gly Ser Cys Ala Tyr Thr Phe Leu Leu Pro
 35 40 45
 Glu Met Asp Asn Gly Arg Ser Ser Ser Thr Tyr Val Thr Asn Ala
 50 55 60
 Val Gln Arg Asp Ala Pro Pro Glu Tyr Glu Asp Ser Val Gln Ser Leu
 65 70 75 80
 Gln Leu Leu Glu Asn Val Met Glu Asn Tyr Thr Gln Trp Leu Met Lys
 85 90 95
 Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Ala Glu Ile
 100 105 110
 Gln Gln Asn Ala Val Gln Asn His Thr Ala Val Met Ile Glu Ile Gly
 115 120 125
 Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
 130 135 140
 Val Glu Thr Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
 145 150 155 160
 Leu Gln His Ser Ile Ser Thr Tyr Lys Leu Glu Lys Gln Ile Leu Asp
 165 170 175
 Gln Thr Ser Glu Ile Asn Lys Ile His Asp Lys Asn Ser Phe Leu Glu
 180 185 190
 Lys Lys Val Leu Asp Met Glu Asp Lys His Ile Ile Glu Met Gln Thr
 195 200 205
 Ile Lys Glu Glu Lys Asp Glu Leu Gln Val Leu Val Ser Lys Gln Asn
 210 215 220
 Ser Ile Ile Glu Glu Leu Glu Lys Ile Val Thr Ala Thr Val Asn
 225 230 235 240
 Asn Ser Val Leu Gln Lys Gln His Asp Leu Met Asp Thr Val Asn
 245 250 255
 Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Ser Thr
 260 265 270
 Val Ala Arg Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Asp Val Phe
 275 280 285
 Lys Ala Gly His Thr Lys Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn
 290 295 300
 Ser Pro Glu Glu Ile Lys Ala Tyr Cys Asn Met Asp Ala Gly Gly Gly
 305 310 315 320
 Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Leu Asp Phe Gln
 325 330 335
 Lys Gly Trp Lys Glu Tyr Lys Val Gly Phe Gly Ser Pro Ser Gly Glu
 340 345 350
 Tyr Trp Leu Gly Asn Glu Phe Ile Ser Gln Ile Thr Asn Gln Gln Arg
 355 360 365
 Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr
 370 375 380
 Ser Leu Tyr Asp His Phe Tyr Ile Ser Gly Glu Glu Leu Asn Tyr Arg
 385 390 395 400
 Ile His Leu Lys Gly Leu Thr Gly Thr Ala Ala Lys Ile Ser Ser Ile
 405 410 415
 Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys
 420 425 430
 Cys Ile Cys Lys Cys Ser Leu Met Leu Thr Gly Gly Trp Trp Phe Asp
 435 440 445
 Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Pro Gln Arg Gln

450	455	460	
Asn Thr Asn Lys Phe Asn	Gly Ile Lys Trp	Tyr Tyr Trp Lys Gly Ser	
465	470	475	480
Gly Tyr Ser Ile Lys Ala Thr Thr Met	Met Ile Arg Pro Ala Asp	Phe	
485	490	495	

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1512 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 1...1509
(C) OTHER INFORMATION:

(A) NAME/KEY: TIE ligand-4

(B) LOCATION: 1...1512
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATG CTC TCC CAG CTA GCC ATG CTG CAG GGC AGC CTC CTC CTT GTG GTT Met Leu Ser Gln Leu Ala Met Leu Gln Gly Ser Leu Leu Leu Val Val	1 5 10 15	48
GCC ACC ATG TCT GTG GCT CAA CAG ACA AGG CAG GAG GCG GAT AGG GGC Ala Thr Met Ser Val Ala Gln Gln Thr Arg Gln Glu Ala Asp Arg Gly	20 25 30	96
TGC GAG ACA CTT GTA GTC CAG CAC GGC CAC TGT AGC TAC ACC TTC TTG Cys Glu Thr Leu Val Val Gln His Gly His Cys Ser Tyr Thr Phe Leu	35 40 45	144
CTG CCC AAG TCT GAG CCC TGC CCT CCG GGG CCT GAG GTC TCC AGG GAC Leu Pro Lys Ser Glu Pro Cys Pro Pro Gly Pro Glu Val Ser Arg Asp	50 55 60	192
TCC AAC ACC CTC CAG AGA GAA TCA CTG GCC AAC CCA CTG CAC CTG GGG Ser Asn Thr Leu Gln Arg Glu Ser Leu Ala Asn Pro Leu His Leu Gly	65 70 75 80	240
AAG TTG CCC ACC CAG CAG GTG AAA CAG CTG GAG CAG GCA CTG CAG AAC Lys Leu Pro Thr Gln Gln Val Lys Gln Leu Glu Gln Ala Leu Gln Asn	85 90 95	288
AAC ACG CAG TGG CTG AAG AAG CTA GAG AGG GCC ATC AAG ACG ATC TTG Asn Thr Gln Trp Leu Lys Lys Leu Glu Arg Ala Ile Lys Thr Ile Leu	100 105 110	336
AGG TCG AAG CTG GAG CAG GTC CAG CAG CAA ATG GCC CAG AAT CAG ACG Arg Ser Lys Leu Glu Gln Val Gln Gln Gln Met Ala Gln Asn Gln Thr	115 120 125	384
GCC CCC ATG CTA GAG CTG GGC ACC AGC CTC CTG AAC CAG ACC ACT GCC Ala Pro Met Leu Glu Leu Gly Thr Ser Leu Leu Asn Gln Thr Thr Ala	130 135 140	432
CAG ATC CGC AAG CTG ACC GAC ATG GAG GCT CAG CTC CTG AAC CAG ACA Gln Ile Arg Lys Leu Thr Asp Met Glu Ala Gln Leu Leu Asn Gln Thr	145 150 155	480

145	150	155	160	
TCA AGA ATG GAT GCC CAG ATG CCA GAG ACC TTT CTG TCC ACC AAC AAG Ser Arg Met Asp Ala Gln Met Pro Glu Thr Phe Leu Ser Thr Asn Lys				528
165	170	175		
CTG GAG AAC CAG CTG CTG CTA CAG AGG CAG AAG CTC CAG CAG CTT CAG Leu Glu Asn Gln Leu Leu Leu Gln Arg Gln Lys Leu Gln Gln Leu Gln				576
180	185	190		
GGC CAA AAC AGC GCG CTC GAG AAG CGG TTG CAG GCC CTG GAG ACC AAG Gly Gln Asn Ser Ala Leu Glu Lys Arg Leu Gln Ala Leu Glu Thr Lys				624
195	200	205		
CAG CAG GAG GAG CTG GCC AGC ATC CTC AGC AAG AAG GCG AAG CTG CTG Gln Gln Glu Glu Leu Ala Ser Ile Leu Ser Lys Lys Ala Lys Leu Leu				672
210	215	220		
AAC ACG CTG AGC CGC CAG AGC GCC GCC CTC ACC AAC ATC GAG CGC GGC Asn Thr Leu Ser Arg Gln Ser Ala Ala Leu Thr Asn Ile Glu Arg Gly				720
225	230	235	240	
CTG CGC GGT GTC AGG CAC AAC TCC AGC CTC CTG CAG GAC CAG CAC Leu Arg Gly Val Arg His Asn Ser Ser Leu Leu Gln Asp Gln Gln His				768
245	250	255		
AGC CTG CGC CAG CTG CTG GTG TTG TTG CGG CAC CTG GTG CAA GAA AGG Ser Leu Arg Gln Leu Leu Val Leu Leu Arg His Leu Val Gln Glu Arg				816
260	265	270		
GCT AAC GCC TCG GCC CCG GCC TTC ATA ATG GCA GGT GAG CAG GTG TTC Ala Asn Ala Ser Ala Pro Ala Phe Ile Met Ala Gly Glu Gln Val Phe				864
275	280	285		
CAG GAC TGT GCA GAG ATC CAG CGC TCT GGG GCC AGT GCC AGT GGT GTC Gln Asp Cys Ala Glu Ile Gln Arg Ser Gly Ala Ser Ala Ser Gly Val				912
290	295	300		
TAC ACC ATC CAG GTG TCC AAT GCA ACG AAG CCC AGG AAG GTG TTC TGT Tyr Thr Ile Gln Val Ser Asn Ala Thr Lys Pro Arg Lys Val Phe Cys				960
305	310	315	320	
GAC CTG CAG AGC AGT GGA GGC AGG TGG ACC CTC ATC CAG CGC CGT GAG Asp Leu Gln Ser Ser Gly Gly Arg Trp Thr Leu Ile Gln Arg Arg Glu				1008
325	330	335		
AAT GGC ACC GTG AAT TTT CAG CGG AAC TGG AAG GAT TAC AAA CAG GGC Asn Gly Thr Val Asn Phe Gln Arg Asn Trp Lys Asp Tyr Lys Gln Gly				1056
340	345	350		
TTC GGA GAC CCA GCT GGG GAG CAC TGG CTG GGC AAT GAA GTG GTG CAC Phe Gly Asp Pro Ala Gly Glu His Trp Leu Gly Asn Glu Val Val His				1104
355	360	365		
CAG CTC ACC AGA AGG GCA GCC TAC TCT CTG CGT GTG GAG CTG CAA GAC Gln Leu Thr Arg Arg Ala Ala Tyr Ser Leu Arg Val Glu Leu Gln Asp				1152
370	375	380		
TGG GAA GGC CAC GAG GCC TAT GCC CAG TAC GAA CAT TTC CAC CTG GGC Trp Glu Gly His Glu Ala Tyr Ala Gln Tyr Glu His Phe His Leu Gly				1200
385	390	395	400	
AGT GAG AAC CAG CTA TAC AGG CTT TCT GTG GTC GGG TAC AGC GGC TCA Ser Glu Asn Gln Leu Tyr Arg Leu Ser Val Val Gly Tyr Ser Gly Ser				1248
405	410	415		
GCA GGG CGC CAG AGC AGC CTG GTC CTG CAG AAC ACC AGC TTT AGC ACC				1296

Ala Gly Arg Gln Ser Ser Leu Val Leu Gln Asn Thr Ser Phe Ser Thr		
420	425	430
CTT GAC TCA GAC AAC GAC CAC TGT CTC TGC AAG TGT GCC CAG GTG ATG		1344
Leu Asp Ser Asp Asn Asp His Cys Leu Cys Lys Cys Ala Gln Val Met		
435	440	445
TCT GGA GGG TGG TGG TTT GAC GCC TGT GGC CTG TCA AAC CTC AAC GGC		1392
Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn Leu Asn Gly		
450	455	460
GTC TAC TAC CAC GCT CCC GAC AAC AAG TAC AAG ATG GAC GGC ATC CGC		1440
Val Tyr Tyr His Ala Pro Asp Asn Lys Tyr Lys Met Asp Gly Ile Arg		
465	470	475
TGG CAC TAC TTC AAG GGC CCC AGC TAC TCA CTG CGT GCC TCT CGC ATG		1488
Trp His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ala Ser Arg Met		
485	490	495
ATG ATA CGG CCT TTG GAC ATC TAA		1512
Met Ile Arg Pro Leu Asp Ile		
500		

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 503 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (v) FRAGMENT TYPE: internal
- (ix) FEATURE:
 - (A) NAME/KEY: TIE ligand-4
 - (B) LOCATION: 1...503
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Leu Ser Gln Leu Ala Met Leu Gln Gly Ser Leu Leu Val Val			
1	5	10	15
Ala Thr Met Ser Val Ala Gln Gln Thr Arg Gln Glu Ala Asp Arg Gly			
20	25	30	
Cys Glu Thr Leu Val Val Gln His Gly His Cys Ser Tyr Thr Phe Leu			
35	40	45	
Leu Pro Lys Ser Glu Pro Cys Pro Pro Gly Pro Glu Val Ser Arg Asp			
50	55	60	
Ser Asn Thr Leu Gln Arg Glu Ser Leu Ala Asn Pro Leu His Leu Gly			
65	70	75	80
Lys Leu Pro Thr Gln Gln Val Lys Gln Leu Glu Gln Ala Leu Gln Asn			
85	90	95	
Asn Thr Gln Trp Leu Lys Lys Leu Glu Arg Ala Ile Lys Thr Ile Leu			
100	105	110	
Arg Ser Lys Leu Glu Gln Val Gln Gln Met Ala Gln Asn Gln Thr			
115	120	125	
Ala Pro Met Leu Glu Leu Gly Thr Ser Leu Leu Asn Gln Thr Thr Ala			
130	135	140	
Gln Ile Arg Lys Leu Thr Asp Met Glu Ala Gln Leu Leu Asn Gln Thr			
145	150	155	160
Ser Arg Met Asp Ala Gln Met Pro Glu Thr Phe Leu Ser Thr Asn Lys			
165	170	175	
Leu Glu Asn Gln Leu Leu Gln Arg Gln Lys Leu Gln Gln Leu Gln			
180	185	190	

Gly Gln Asn Ser Ala Leu Glu Lys Arg Leu Gln Ala Leu Glu Thr Lys
 195 200 205
 Gln Gln Glu Glu Leu Ala Ser Ile Leu Ser Lys Lys Ala Lys Leu Leu
 210 215 220
 Asn Thr Leu Ser Arg Gln Ser Ala Ala Leu Thr Asn Ile Glu Arg Gly
 225 230 235 240
 Leu Arg Gly Val Arg His Asn Ser Ser Leu Leu Gln Asp Gln Gln His
 245 250 255
 Ser Leu Arg Gln Leu Leu Val Leu Leu Arg His Leu Val Gln Glu Arg
 260 265 270
 Ala Asn Ala Ser Ala Pro Ala Phe Ile Met Ala Gly Glu Gln Val Phe
 275 280 285
 Gln Asp Cys Ala Glu Ile Gln Arg Ser Gly Ala Ser Ala Ser Gly Val
 290 295 300
 Tyr Thr Ile Gln Val Ser Asn Ala Thr Lys Pro Arg Lys Val Phe Cys
 305 310 315 320
 Asp Leu Gln Ser Ser Gly Gly Arg Trp Thr Leu Ile Gln Arg Arg Glu
 325 330 335
 Asn Gly Thr Val Asn Phe Gln Arg Asn Trp Lys Asp Tyr Lys Gln Gly
 340 345 350
 Phe Gly Asp Pro Ala Gly Glu His Trp Leu Gly Asn Glu Val Val His
 355 360 365
 Gln Leu Thr Arg Arg Ala Ala Tyr Ser Leu Arg Val Glu Leu Gln Asp
 370 375 380
 Trp Glu Gly His Glu Ala Tyr Ala Gln Tyr Glu His Phe His Leu Gly
 385 390 395 400
 Ser Glu Asn Gln Leu Tyr Arg Leu Ser Val Val Gly Tyr Ser Gly Ser
 405 410 415
 Ala Gly Arg Gln Ser Ser Leu Val Leu Gln Asn Thr Ser Phe Ser Thr
 420 425 430
 Leu Asp Ser Asp Asn Asp His Cys Leu Cys Lys Cys Ala Gln Val Met
 435 440 445
 Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn Leu Asn Gly
 450 455 460
 Val Tyr Tyr His Ala Pro Asp Asn Lys Tyr Lys Met Asp Gly Ile Arg
 465 470 475 480
 Trp His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ala Ser Arg Met
 485 490 495
 Met Ile Arg Pro Leu Asp Ile
 500

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1497 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...1494

(D) OTHER INFORMATION:

(A) NAME/KEY: 1N1C2F (chimera 1)

(B) LOCATION: 1...1497

(D) OTHER INFORMATION:

(A) NAME/KEY: Other

(B) LOCATION: 1...60

(D) OTHER INFORMATION: Putative leader sequence is encoded by nucleotides 1-60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATG ACA GTT TTC CTT TCC TTT GCT TTC CTC GCT GCC ATT CTG ACT CAC Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His 1 5 10 15	48
ATA GGG TGC AGC AAT CAG CGC CGA AGT CCA GAA AAC AGT GGG AGA AGA Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg 20 25 30	96
TAT AAC CGG ATT CAA CAT GGG CAA TGT GCC TAC ACT TTC ATT CTT CCA Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro 35 40 45	144
GAA CAC GAT GGC AAC TGT CGT GAG AGT ACG ACA GAC CAG TAC AAC ACA Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr 50 55 60	192
AAC GCT CTG CAG AGA GAT GCT CCA CAC GTG GAA CCG GAT TTC TCT TCC Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser 65 70 75 80	240
CAG AAA CTT CAA CAT CTG GAA CAT GTG ATG GAA AAT TAT ACT CAG TGG Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp 85 90 95	288
CTG CAA AAA CTT GAG AAT TAC ATT GTG GAA AAC ATG AAG TCG GAG ATG Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met 100 105 110	336
GCC CAG ATA CAG CAG AAT GCA GTT CAG AAC CAC ACG GCT ACC ATG CTG Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu 115 120 125	384
GAG ATA GGA ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC AGA AAG Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys 130 135 140	432
CTG ACA GAT GTT GAG ACC CAG GTA CTA AAT CAA ACT TCT CGA CTT GAG Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu 145 150 155 160	480
ATA CAG CTG CTG GAG AAT TCA TTA TCC ACC TAC AAG CTA GAG AAG CAA Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln 165 170 175	528
CTT CTT CAA CAG ACA AAT GAA ATC TTG AAG ATC CAT GAA AAA AAC AGT Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser 180 185 190	576
TTA TTA GAA CAT AAA ATC TTA GAA ATG GAA GGA AAA CAC AAG GAA GAG Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu 195 200 205	624
TTG GAC ACC TTA AAG GAA GAG AAA GAG AAC CTT CAA GGC TTG GTT ACT Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr 210 215 220	672
CGT CAA ACA TAT ATA ATC CAG GAG CTG GAA AAG CAA TTA AAC AGA GCT Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala 225 230 235 240	720
ACC ACC AAC AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG ATG GAC Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp 245 250 255	768
ACA GTC CAC AAC CTT GTC AAT CTT TGC ACT AAA GAA GGT GTT TTA CTA	816

Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu		
260	265	270
AAG GGA GGA AAA AGA GAG GAA GAG AAA CCA TTT AGA GAC TGT GCT GAA		864
Lys Gly Gly Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala Glu		
275	280	285
GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA ACA TTC		912
Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe		
290	295	300
CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT GGA		960
Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly		
305	310	315
GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC AGC GTC GAT		1008
Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp		
325	330	335
TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT TCA		1056
Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser		
340	345	350
GGA GAA TAT TGG CTG GGA AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG		1104
Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln		
355	360	365
CAA CGC TAT GTG CTT AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG		1152
Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu		
370	375	380
GCT TAC TCA TTG TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT		1200
Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn		
385	390	395
TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC		1248
Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser		
405	410	415
AGC ATC AGC CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC		1296
Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn		
420	425	430
GAC AAA TGT ATT TGC AAA TGT TCA CAA ATC CTA ACA GGA GGC TGG TGG		1344
Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp		
435	440	445
TTT GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG		1392
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln		
450	455	460
AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG AAA		1440
Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys		
465	470	475
GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA		1488
Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala		
485	490	495
GAT TTC TAA		1497
Asp Phe		

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LEN TH: 498 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (v) FRAGMENT TYPE: internal
 (ix) FEATURE:

(A) NAME/KEY: 1N1C2F (chimera 1)
 (B) LOCATION: 1...498
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
 1 5 10 15
 Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
 20 25 30
 Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
 35 40 45
 Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
 50 55 60
 Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
 65 70 75 80
 Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
 85 90 95
 Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
 100 105 110
 Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
 115 120 125
 Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
 130 135 140
 Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
 145 150 155 160
 Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
 165 170 175
 Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
 180 185 190
 Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
 195 200 205
 Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
 210 215 220
 Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
 225 230 235 240
 Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
 245 250 255
 Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu
 260 265 270
 Lys Gly Gly Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala Glu
 275 280 285
 Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe
 290 295 300
 Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly
 305 310 315 320
 Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp
 325 330 335
 Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser
 340 345 350
 Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln
 355 360 365
 Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu
 370 375 380
 Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn
 385 390 395 400
 Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser

405	410	415
Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn		
420	425	430
Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp		
435	440	445
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln		
450	455	460
Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys		
465	470	475
Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala		
485	490	495
Asp Phe		

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1491 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...1488
- (D) OTHER INFORMATION:

- (A) NAME/KEY: 2N2C1F (chimera 2)
- (B) LOCATION: 1...1491
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...48
- (D) OTHER INFORMATION: Putative leader sequence is encoded by nucleotides 1-48

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATG TGG CAG ATT GTT TTC TTT ACT CTG AGC TGT GAT CTT GTC TTG GCC	48
Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala	
1 5 10 15	
GCA GCC TAT AAC AAC TTT CGG AAG AGC ATG GAC AGC ATA GGA AAG AAG	96
Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys	
20 25 30	
CAA TAT CAG GTC CAG CAT GGG TCC TGC AGC TAC ACT TTC CTC CTG CCA	144
Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro	
35 40 45	
GAG ATG GAC AAC TGC CGC TCT TCC TCC AGC CCC TAC GTG TCC AAT GCT	192
Glu Met Asp Asn Cys Arg Ser Ser Ser Pro Tyr Val Ser Asn Ala	
50 55 60	
GTG CAG AGG GAC GCG CCG CTC GAA TAC GAT GAC TCG GTG CAG AGG CTG	240
Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu	
65 70 75 80	
CAA GTG CTG GAG AAC ATC ATG GAA AAC AAC ACT CAG TGG CTA ATG AAG	288
Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys	
85 90 95	
CTT GAG AAT TAT ATC CAG GAC AAC ATG AAG AAA GAA ATG GTA GAG ATA	336

Leu	Glu	Asn	Tyr	Ile	Gln	Asp	Asn	Met	Lys	Glu	Met	Val	Glu	Ile
								100	105				110	
CAG	CAG	AAT	GCA	GTA	CAG	AAC	CAG	ACG	GCT	GTG	ATG	ATA	GAA	ATA
Gln	Gln		Asn	Ala	Val	Gln	Asn	Gln	Thr	Ala	Val	Met	Ile	Glu
														Gly
115							120						125	
ACA	AAC	CTG	TTG	AAC	CAA	ACA	GCT	GAG	CAA	ACG	CGG	AAG	TTA	ACT
Thr	Asn	Leu	Leu	Asn	Gln	Thr	Ala	Glu	Gln	Thr	Arg	Lys	Leu	Thr
														Asp
130							135				140			
GTG	GAA	GCC	CAA	GTA	TTA	AAT	CAG	ACC	ACG	AGA	CTT	GAA	CTT	CAG
Val	Glu	Ala	Gln	Val	Leu	Asn	Gln	Thr	Thr	Arg	Leu	Glu	Leu	Gln
														Leu
145							150				155			160
TTG	GAA	CAC	TCC	CTC	TCG	ACA	AAC	AAA	TTG	GAA	AAA	CAG	ATT	TTG
Leu	Glu	His	Ser	Leu	Ser	Thr	Asn	Lys	Leu	Glu	Lys	Gln	Ile	Leu
														Asp
165							170						175	
CAG	ACC	AGT	GAA	ATA	AAC	AAA	TTG	CAA	GAT	AAG	AAC	AGT	TTC	CTA
Gln	Thr	Ser	Glu	Ile	Asn	Lys	Leu	Gln	Asp	Lys	Asn	Ser	Phe	Leu
														Glu
180							185						190	
AAG	AAG	GTG	CTA	GCT	ATG	GAA	GAC	AAG	CAC	ATC	ATC	CAA	CTA	CAG
Lys	Lys	Val	Leu	Ala	Met	Glu	Asp	Lys	His	Ile	Ile	Gln	Leu	Gln
														Ser
195							200						205	
ATA	AAA	GAA	GAG	AAA	GAT	CAG	CTA	CAG	GTG	TTA	GTA	TCC	AAG	CAA
Ile	Lys	Glu	Glu	Lys	Asp	Gln	Leu	Gln	Val	Leu	Val	Ser	Lys	Gln
														Asn
210							215						220	
TCC	ATC	ATT	GAA	GAA	CTA	GAA	AAA	AAA	ATA	GTG	ACT	GCC	ACG	GTG
Ser	Ile	Ile	Glu	Glu	Leu	Glu	Lys	Lys	Ile	Val	Thr	Ala	Thr	Val
														Asn
225							230				235			240
AAT	TCA	GTT	CTT	CAA	AAG	CAG	CAA	CAT	GAT	CTC	ATG	GAG	ACA	GTT
Asn	Ser	Val	Leu	Gln	Lys	Gln	Gln	His	Asp	Leu	Met	Glu	Thr	Val
														Asn
245							250						255	
AAC	TTA	CTG	ACT	ATG	ATG	TCC	ACA	TCA	AAC	TCA	GCT	AAG	GAC	CCC
Asn	Leu	Leu	Thr	Met	Met	Ser	Thr	Ser	Asn	Ser	Ala	Lys	Asp	Pro
														Thr
260							265						270	
GTT	GCT	AAA	GAA	GAA	CAA	ATC	AGC	TTC	AGA	GAC	TGT	GCA	GAT	GTA
Val	Ala	Lys	Glu	Glu	Gln	Ile	Ser	Phe	Arg	Asp	Cys	Ala	Asp	Val
														Tyr
275							280						285	
CAA	GCT	GGT	TTT	AAT	AAA	AGT	GGA	ATC	TAC	ACT	ATT	TAT	ATT	AAT
Gln	Ala	Gly	Phe	Asn	Lys	Ser	Gly	Ile	Tyr	Thr	Ile	Tyr	Ile	Asn
														Asn
290							295						300	
ATG	CCA	GAA	CCC	AAA	AAG	GTG	TTT	TGC	AAT	ATG	GAT	GTC	AAT	GGG
Met	Pro	Glu	Pro	Lys	Lys	Val	Phe	Cys	Asn	Met	Asp	Val	Asn	Gly
														Gly
305							310				315			320
GGT	TGG	ACT	GTA	ATA	CAA	CAT	CGT	GAA	GAT	GGA	AGT	CTA	GAT	TTC
Gly	Trp	Thr	Val	Ile	Gln	His	Arg	Glu	Asp	Gly	Ser	Leu	Asp	Phe
														Gln
325							330						335	
AGA	GGC	TGG	AAG	GAA	TAT	AAA	ATG	GGT	TTT	GGA	AAT	CCC	TCC	GGT
Arg	Gly	Trp	Lys	Glu	Tyr	Lys	Met	Gly	Phe	Gly	Asn	Pro	Ser	Gly
														Glu
340							345						350	
TAT	TGG	CTG	GGG	AAT	GAG	TTT	ATT	TTT	GCC	ATT	ACC	AGT	CAG	AGG
Tyr	Trp	Leu	Gly	Asn	Glu	Tyr	Lys	Met	Ile	Phe	Ala	Ile	Thr	Ser
														Gln
355							360						365	

TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT	1152
Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr	
370 375 380	
TCA CAG TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG	1200
Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg	
385 390 395 400	
TTG TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG	1248
Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu	
405 410 415	
ATC TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC	1296
Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn	
420 425 430	
TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT	1344
Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp	
435 440 445	
GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA	1392
Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln	
450 455 460	
AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC	1440
Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro	
465 470 475 480	
AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT T	1489
Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe	
485 490 495	

GA

1491

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 496 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: 2N2C1F (chimera 2)

(B) LOCATION: 1...496

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala	
1 5 10 15	
Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys	
20 25 30	
Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro	
35 40 45	
Glu Met Asp Asn Cys Arg Ser Ser Ser Pro Tyr Val Ser Asn Ala	
50 55 60	
Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu	
65 70 75 80	
Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys	
85 90 95	
Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile	
100 105 110	

Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
 115 120 125
 Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
 130 135 140
 Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
 145 150 155 160
 Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
 165 170 175
 Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
 180 185 190
 Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
 195 200 205
 Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
 210 215 220
 Ser Ile Ile Glu Glu Leu Glu Lys Ile Val Thr Ala Thr Val Asn
 225 230 235 240
 Asn Ser Val Leu Gln Lys Gln His Asp Leu Met Glu Thr Val Asn
 245 250 255
 Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
 260 265 270
 Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Asp Val Tyr
 275 280 285
 Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn
 290 295 300
 Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly
 305 310 315 320
 Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln
 325 330 335
 Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu
 340 345 350
 Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln
 355 360 365
 Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr
 370 375 380
 Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg
 385 390 395 400
 Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu
 405 410 415
 Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn
 420 425 430
 Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp
 435 440 445
 Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln
 450 455 460
 Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro
 465 470 475 480
 Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
 485 490 495

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence

- (B) LOCATION: 1...1497

- (D) OTHER INFORMATION:

- (A) NAME/KEY: 1N2C2F (chimera 3)

- (B) LOCATION: 1...1500

(D) OTHER INFORMATION:

(A) NAME/KEY: Other
 (B) LOCATION: 1...60
 (D) OTHER INFORMATION: Putative leader sequence is encoded by nucleotides 1-60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATG ACA GTT TTC CTT TCC TTT GCT TTC CTC GCT GCC ATT CTG ACT CAC Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His 1 5 10 15	48
ATA GGG TGC AGC AAT CAG CGC CGA AGT CCA GAA AAC AGT GGG AGA AGA Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg 20 25 30	96
TAT AAC CGG ATT CAA CAT GGG CAA TGT GCC TAC TTC ATT CTT CCA Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro 35 40 45	144
GAA CAC GAT GCC AAC TGT CGT GAG AGT ACG ACA GAC CAG TAC AAC ACA Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr 50 55 60	192
AAC GCT CTG CAG AGA GAT GCT CCA CAC GTG GAA CCG GAT GAC TCG GTG Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Asp Ser Val 65 70 75 80	240
CAG AGG CTG CAA GTG CTG GAG AAC ATC ATG GAA AAC AAC ACT CAG TGG Gln Arg Leu Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp 85 90 95	288
CTA ATG AAG CTT GAG AAT TAT ATC CAG GAC AAC ATG AAG AAA GAA ATG Leu Met Lys Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met 100 105 110	336
GTA GAG ATA CAG CAG AAT GCA GTA CAG AAC CAG ACG GCT GTG ATG ATA Val Glu Ile Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile 115 120 125	384
GAA ATA GGG ACA AAC CTG TTG AAC CAA ACA GCT GAG CAA ACG CGG AAG Glu Ile Gly Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys 130 135 140	432
TTA ACT GAT GTG GAA GCC CAA GTA TTA AAT CAG ACC ACG AGA CTT GAA Leu Thr Asp Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu 145 150 155 160	480
CTT CAG CTC TTG GAA CAC TCC CTC TCG ACA AAC AAA TTG GAA AAA CAG Leu Gln Leu Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln 165 170 175	528
ATT TTG GAC CAG ACC AGT GAA ATA AAC AAA TTG CAA GAT AAG AAC AGT Ile Leu Asp Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser 180 185 190	576
TTC CTA GAA AAG AAG GTG CTA GCT ATG GAA GAC AAG CAC ATC ATC CAA Phe Leu Glu Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln 195 200 205	624
CTA CAG TCA ATA AAA GAA GAG AAA GAT CAG CTA CAG GTG TTA GTA TCC Leu Gln Ser Ile Lys Glu Lys Asp Gln Leu Gln Val Leu Val Ser 210 215 220	672
AAG CAA AAT TCC ATC ATT GAA GAA CTA GAA AAA AAA ATA GTG ACT GCC	720

Lys Gln Asn Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala			
225	230	235	240
ACG GTG AAT AAT TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC ATG GAG			768
Thr Val Asn Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu			
245	250	255	
ACA GTT AAT AAC TTA CTG ACT ATG ATG TCC ACA TCA AAC TCA GCT AAG			816
Thr Val Asn Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys			
260	265	270	
GAC CCC ACT GTT GCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCT			864
Asp Pro Thr Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala			
275	280	285	
GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA ACA			912
Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr			
290	295	300	
TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT			960
Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala			
305	310	315	320
GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC AGC GTT			1008
Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val			
325	330	335	
GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT			1056
Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro			
340	345	350	
TCA GGA GAA TAT TGG CTG GGA AAT GAG TTT GTT TCG CAA CTG ACT AAT			1104
Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn			
355	360	365	
CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT			1152
Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn			
370	375	380	
GAG GCT TAC TCA TTG TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC			1200
Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu			
385	390	395	400
AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA			1248
Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile			
405	410	415	
AGC AGC ATC AGC CAA CCA GGA AAT CAT TTT AGC ACA AAG GAT GGA GAC			1296
Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp			
420	425	430	
AAC GAC AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG			1344
Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp			
435	440	445	
TGG TTT GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA			1392
Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro			
450	455	460	
CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG			1440
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp			
465	470	475	480
AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA			1488
Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro			
485	490	495	

GCA GAT TTC TAA
Ala Asp Phe

1500

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 499 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: 1N2C2F (chimera 3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
 1 5 10 15
 Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
 20 25 30
 Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
 35 40 45
 Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
 50 55 60
 Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Asp Ser Val
 65 70 75 80
 Gln Arg Leu Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp
 85 90 95
 Leu Met Lys Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met
 100 105 110
 Val Glu Ile Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile
 115 120 125
 Glu Ile Gly Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys
 130 135 140
 Leu Thr Asp Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu
 145 150 155 160
 Leu Gln Leu Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln
 165 170 175
 Ile Leu Asp Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser
 180 185 190
 Phe Leu Glu Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln
 195 200 205
 Leu Gln Ser Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser
 210 215 220
 Lys Gln Asn Ser Ile Ile Glu Glu Leu Glu Lys Ile Val Thr Ala
 225 230 235 240
 Thr Val Asn Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu
 245 250 255
 Thr Val Asn Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys
 260 265 270
 Asp Pro Thr Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala
 275 280 285
 Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr
 290 295 300
 Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala
 305 310 315 320
 Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val
 325 330 335
 Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro
 340 345 350
 Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn
 355 360 365

Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn
 370 375 380
 Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu
 385 390 395 400
 Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile
 405 410 415
 Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp
 420 425 430
 Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp
 435 440 445
 Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro
 450 455 460
 Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp
 465 470 475 480
 Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro
 485 490 495
 Ala Asp Phe

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1488 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...1485
- (D) OTHER INFORMATION:

- (A) NAME/KEY: 2N1C1F (chimera 4)
- (B) LOCATION: 1...1488
- (D) OTHER INFORMATION:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...48
- (D) OTHER INFORMATION: Putative leader sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATG TGG CAG ATT GTT TTC TTT ACT CTG AGC TGT GAT CTT GTC TTG GCC	48
Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala	
1 5 10 15	
GCA GCC TAT AAC AAC TTT CGG AAG AGC ATG GAC AGC ATA GGA AAG AAG	96
Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys	
20 25 30	
CAA TAT CAG GTC CAG CAT GGG TCC TGC AGC TAC ACT TTC CTC CTG CCA	144
Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro	
35 40 45	
GAG ATG GAC AAC TGC CGC TCT TCC TCC AGC CCC TAC GTG TCC AAT GCT	192
Glu Met Asp Asn Cys Arg Ser Ser Ser Pro Tyr Val Ser Asn Ala	
50 55 60	
GTG CAG AGG GAC GCG CCG CTC GAA TAC GAT TTC TCT TCC CAG AAA CTT	240
Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Phe Ser Ser Gln Lys Leu	
65 70 75 80	

CAA CAT CTG GAA CAT GTG ATG GAA AAT TAT ACT CAG TGG CTG CAA AAA Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys 85 90 95	288
CTT GAG AAT TAC ATT GTG GAA AAC ATG AAG TCG GAG ATG GCC CAG ATA Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile 100 105 110	336
CAG CAG AAT GCA GTT CAG AAC CAC ACG GCT ACC ATG CTG GAG ATA GGA Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly 115 120 125	384
ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC AGA AAG CTG ACA GAT Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp 130 135 140	432
GTT GAG ACC CAG GTA CTA AAT CAA ACT TCT CGA CTT GAG ATA CAG CTG Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu 145 150 155 160	480
CTG GAG AAT TCA TTA TCC ACC TAC AAG CTA GAG AAG CAA CTT CTT CAA Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln 165 170 175	528
CAG ACA AAT GAA ATC TTG AAG ATC CAT GAA AAA AAC ACT TTA TTA GAA Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu 180 185 190	576
CAT AAA ATC TTA GAA ATG GAA GGA AAA CAC AAG GAA GAG TTG GAC ACC His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Leu Asp Thr 195 200 205	624
TTA AAG GAA GAG AAA GAG AAC CTT CAA GCC TTG GTT ACT CGT CAA ACA Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr 210 215 220	672
TAT ATA ATC CAG GAG CTG GAA AAG CAA TTA AAC AGA GCT ACC ACC AAC Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn 225 230 235 240	720
AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG ATG GAC ACA GTC CAC Asn Ser Val Leu Gln Lys Gln Leu Glu Leu Met Asp Thr Val His 245 250 255	768
AAC CTT GTC AAT CTT TGC ACT AAA GAA GGT GTT TTA CTA AAG GGA GGA Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu Lys Gly Gly 260 265 270	816
AAA AGA GAG GAA GAG AAA CCA TTT AGA GAC TGT GCA GAT GTA TAT CAA Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln 275 280 285	864
GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT ATT AAT AAT ATG Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met 290 295 300	912
CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly 305 310 315 320	960
TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA AGT CTA GAT TTC CAA AGA Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg 325 330 335	1008
GGC TGG AAG GAA TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr 340 345 350	1056

TGG CTG GGG AAT GAG TTT ATT TTT GCC ATT ACC AGT CAG AGG CAG TAC Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr 355 360 365	1104
ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser 370 375 380	1152
CAG TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu 385 390 395 400	1200
TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile 405 410 415	1248
TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys 420 425 430	1296
ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT Met Cys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala 435 440 445	1344
TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn 450 455 460	1392
CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC AGT His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser 465 470 475 480	1440
TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT TGA Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe 485 490 495	1488

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 495 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: 2N1C1F (chimera 4)

(B) LOCATION: 1...495

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1 5 10 15
Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
20 25 30
Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
35 40 45
Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
50 55 60
Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Phe Ser Ser Gln Lys Leu
65 70 75 80
Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys

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85	90	95
Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile		
100 105 110		
Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly		
115 120 125		
Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp		
130 135 140		
Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu		
145 150 155 160		
Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln		
165 170 175		
Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu		
180 185 190		
His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Leu Asp Thr		
195 200 205		
Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr		
210 215 220		
Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn		
225 230 235 240		
Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His		
245 250 255		
Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu Lys Gly Gly		
260 265 270		
Lys Arg Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln		
275 280 285		
Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met		
290 295 300		
Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly		
305 310 315 320		
Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg		
325 330 335		
Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr		
340 345 350		
Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr		
355 360 365		
Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser		
370 375 380		
Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu		
385 390 395 400		
Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile		
405 410 415		
Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys		
420 425 430		
Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala		
435 440 445		
Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn		
450 455 460		
His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser		
465 470 475 480		
Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe		
485 490 495		

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: hTL4atg
- (B) LOCATION: 1...47
- (D) OTHER INFORMATION: PCR primer

- (A) NAME/KEY: Other
- (B) LOCATION: 1...20
- (D) OTHER INFORMATION: "tail" sequences added to PCR primer to facilitate cloning of the amplified PCR fragments

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCATGCTATC TCGAGCCACC ATGCTCTCCC AGCTAGCCAT GCTGCAG

47

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 55 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: hTL4not
- (B) LOCATION: 1...55
- (D) OTHER INFORMATION: PCR Primer

- (A) NAME/KEY: Other
- (B) LOCATION: 1...28
- (D) OTHER INFORMATION: "tail" sequence added to the PCR primers to facilitate cloning of the amplified PCR fragments

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTGTCGACGC GGCCGCTCTA GATCAGACTT AGATGTCCAA AGGCCGTATC ATCAT

55

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
 (PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 102, lines 5-19.	
B. IDENTIFICATION OF DEPOSIT	
<small>Further deposits are identified on an additional sheet</small> <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 12301 Parklawn Drive Rockville, Maryland 20852 U.S.A.	
Date of deposit October 7, 1994	Accession Number 75910
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Applicant wishes that, until publication of the mention of the grant of a European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, the deposit shall be made available as provided in Rule 28(3) of the Implementing Regulations under the European Patent Convention only by the issue of a sample to an expert nominated by the requester (Rule 28(4) of the implementing regulations).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., 'Accession Number of Deposit')	
For receiving Office use only <input type="checkbox"/> This sheet was received with the international application	
For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer	

Att. Dkt. No. - REG 333-PCT

Internat'l Applic. No.: NOT YET KNOWN

Internat'l Filing Date: FILED HEREWITH

Title: NOVEL MODIFIED LIGANDS

SUPPLEMENTAL SHEET TO BOX B OF FORM PCT/RO/134

Identification of Further Deposits - In addition to the deposit indicated on the attached Form PCT/RO/134, applicant identifies the following deposits made with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, U.S.A. And requests that they also be made available only by the issue of a sample to an expert nominated by the requester as indicated on the attached form:

<u>Date of Deposit</u>	<u>Accession Number</u>
October 7, 1994	VR2484
October 26, 1994	75928
December 9, 1994	75963
July 2, 1996	90895

What is claimed is:

1. An isolated nucleic acid molecule encoding a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first, wherein the first and second TIE-2 ligands are selected from TIE-2 Ligand 1, TIE-2 Ligand 2, TIE Ligand 3 and TIE Ligand 4.
2. A nucleic acid molecule of claim 1, encoding a chimeric TIE-2 ligand comprising at least a portion of TIE-2 Ligand-1 and a portion of TIE-2 Ligand 2.
3. A nucleic acid molecule according to claim 2, encoding a chimeric TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2.
4. A nucleic acid molecule of claim 3, wherein the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled-coil-domain of TIE-2 ligand 2.
5. A nucleic acid molecule of claim 3 or 4, which is modified

to encode a different amino acid instead of the cysteine residue encoded by nucleotides 784-787 as set forth in Figure 27.

6. A nucleic acid molecule of claim 5, which is modified such that a serine residue is encoded instead of the cysteine residue.
7. A nucleic acid molecule of claim 5 or 6, which is further modified to encode a different amino acid instead of the arginine residue encoded by nucleotides 199-201 as set forth in Figure 27.
8. A nucleic acid molecule of claim 7 which is modified such that a serine residue is encoded instead of the arginine residue.
9. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 which is modified to encode a different amino acid instead of the cysteine residue at amino acid position 245.
10. A nucleic acid molecule of claim 9, which is modified such that a serine residue is encoded instead of the cysteine residue.
11. A nucleic acid molecule of claim 3, having the sequence set

forth in Figure 27.

12. A nucleic acid molecule of claim 4, having the sequence set forth in Figure 25.
13. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 or TIE-2 ligand 2 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 or TIE-2 ligand 2 is deleted.
14. A nucleic acid molecule of claim 13, wherein the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 or TIE-2 ligand 2 is deleted and the portion encoding the fibrinogen-like domain is fused in-frame to a nucleotide sequence encoding a human immunoglobulin gamma-1 constant region (IgG1 Fc).
15. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 2.
16. The nucleic acid molecule of claim 15, wherein the portion

of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled coil domain of TIE-2 ligand 2

17. A nucleic acid molecule of claim 15, having the sequence set forth in Figure 24.
18. A nucleic acid molecule of claim 16, having the sequence set forth in Figure 26.
19. A chimeric or modified TIE-2 ligand encoded by a nucleic acid molecule of any one of the preceding claims.
20. A chimeric TIE ligand according to claim 19, having the sequence set forth in Figure 24, 25, 26 or 27.
21. A chimeric TIE ligand according to claim 19, having the sequence set forth in Figure 27, but modified to have a different amino acid instead of the cysteine residue encoded by nucleotides 784-787
22. A vector which comprises a nucleic acid molecule of any one of preceding claims 1 to 18.
23. A vector according to claim 22, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.

24. A vector according to claim 22 or 23 which is a plasmid.
25. A host-vector system for the production of a chimeric or modified ligand according to any one of claims 19, 20 or 21 which comprises a vector according to any one of claims 22, 23 or 24.
26. A host-vector system according to claim 25 wherein the host cell is a bacterial, yeast, insect or mammalian cell.
27. A method of producing a ligand as defined in claim any one of claims 19, 20 or 21, which comprises growing cells of a host-vector system according to claim 25 or 26, under conditions permitting production of the ligand and recovering the ligand so produced.
28. An antibody which specifically binds the ligand of any one of claims 19, 20 or 21
29. An antibody according to claim 28 which is a monoclonal antibody.
30. A receptorbody which specifically binds the ligand of claim 19, 20 or 21.
31. An isolated nucleic acid molecule encoding a receptorbody according to claim 30.

32. A vector comprising a nucleic acid molecule according to claim 31.
33. A vector according to claim 32 which is a plasmid.
34. A conjugate comprising a ligand according to claim any one of claims 19, 20 or 21 and conjugated thereto, a cytotoxic agent.
35. A conjugate according to claim 34 wherein the cytotoxic agent is a radioisotope or toxin.
36. A pharmaceutical composition comprising a chimeric or modified ligand according to any one of claims 19, 20 or 21 and a pharmaceutically acceptable carrier.
37. A pharmaceutical composition comprising an antibody according to claim 28 or 29 and a pharmaceutically acceptable carrier.
38. A pharmaceutical composition comprising a receptorbody according to 30 and a pharmaceutically acceptable carrier.
39. A pharmaceutical composition comprising a conjugate according to 34 or 35 and a pharmaceutically acceptable carrier.

40. A ligand according to any one of claims 19, 20 or 21 an antibody according to claim 28 or 29, a receptorbody according to claim 30 or a conjugate according to claim 34 or 35 for use in a method of treatment of the human or animal body, or in a method of diagnosis.
41. A ligand produced by the method of claim 27.
42. An isolated nucleic acid molecule of claim 1, 9, 13 or 15 substantially as hereinbefore described.
43. A chimeric or modified TIE-2 ligand of claim 19 substantially as hereinbefore described.
44. A vector of claim 22 or 32 substantially as hereinbefore described.
45. A host-vector system of claim 25 substantially as hereinbefore described.
46. A method of claim 27 substantially as hereinbefore described.
47. An antibody of claim 28 substantially as hereinbefore described.
48. A receptorbody of claim 30 substantially as hereinbefore described.

49. A pharmaceutical composition of claim 36, 37, 38 or 39 substantially as hereinbefore described.
50. A ligand, antibody, receptorbody or conjugate of claim 40 substantially as hereinbefore described.

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Fig.1A.



r EHK-1 ecto/h IgG1 Fc
Gelfoam (6ug)

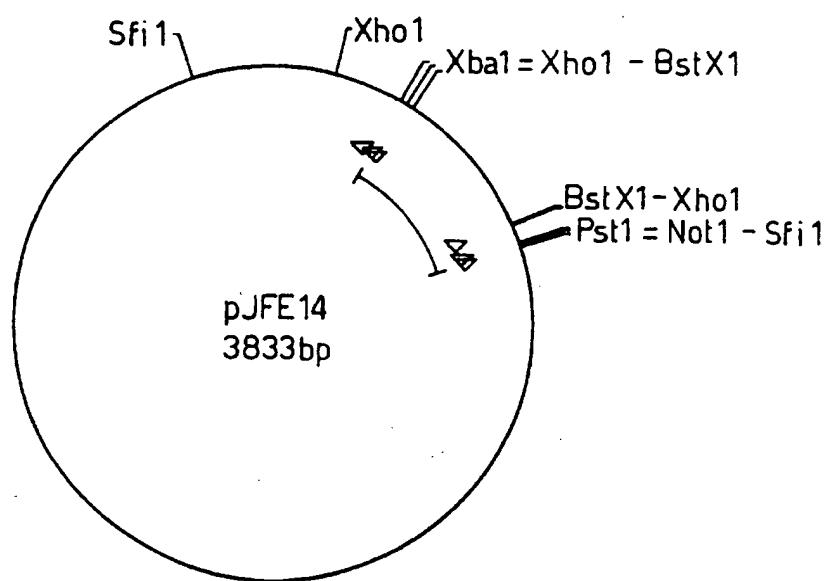
Fig.1B.



r TIE-2 ecto/h IgG1 Fc
Gelfoam (6ug)

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Fig.2.



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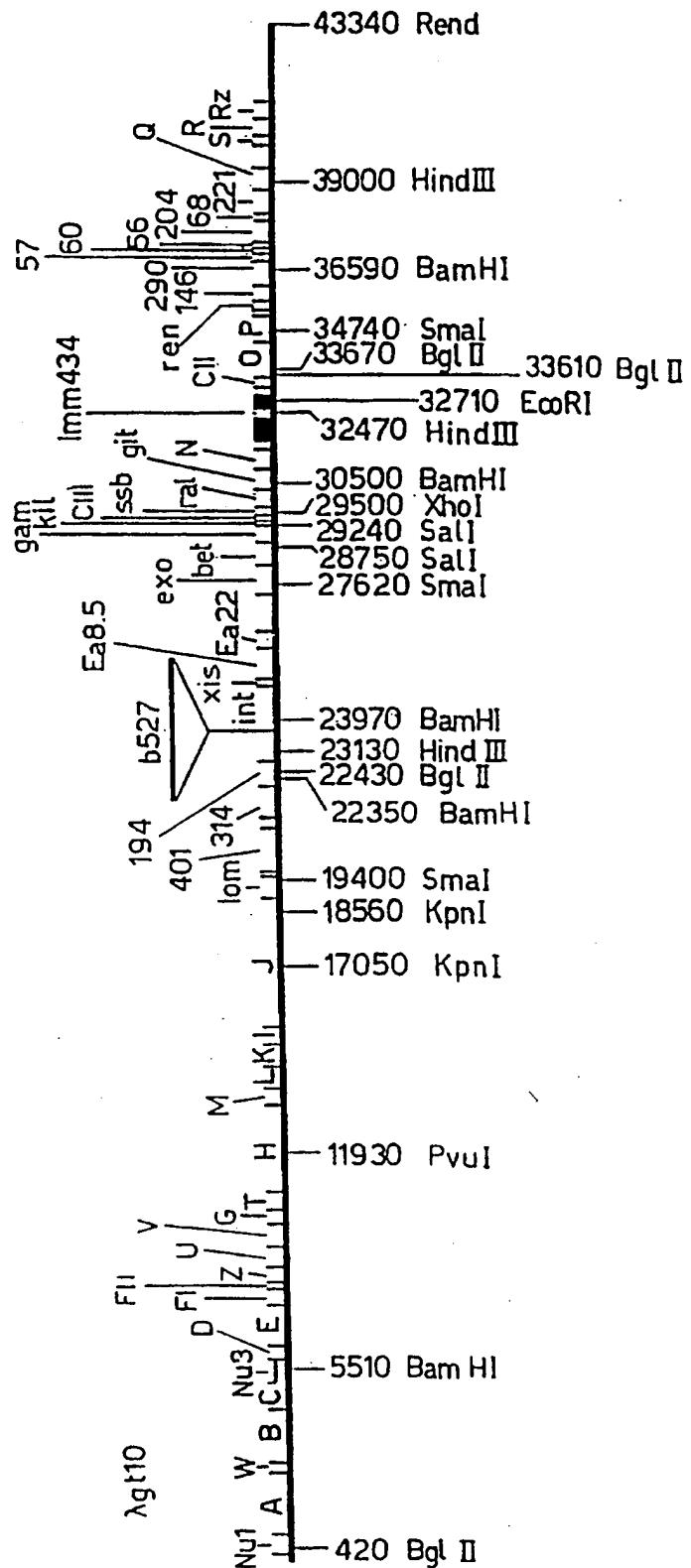


Fig. 3.

Fig.4.

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10 20 30 40 50 60 70 80
 CAGCTGACTCAGGCAGGCTCCATGGTGAACGGTCACACAGAGAGGAACAAATAATCTAGCTACTATGCAATAATATC

90 100 110 120 130 140 150 160
 TCAAGTTTAACGAAGAAAAACATCATGGTGAATAAATTTAAAGAGCTAACAAACAGACTTTACCTGAATAAAGAA

170 180 190 200 210 220 230 240
 TTTCTATGATCTCTCAACGCTTCTTGAGGGGAAAGAGTCAAACAAACAGACTTTACCTGAATAAAGAA

250 260 270 280 290 300 310
 CTAGTTTAGAGGTCAAGAAGAAAGGACCAAGTTTCCGAGAGGCACCGAAGGAGTGTGCTGGCACTACA ATG ACA
 H T>

320 330 340 350 360 370
 GTT TTC CTT TCC TTT CCT TTC CTC GCT GCC ATT CTG ACT CAC ATA CGG TGC AGC AAT CAG
 V F L S F A F L A A I L T H I G C S N Q>

380 390 400 410 420 430
 CGC CGA AGT CCA GAA AAC AGT GGG AGA AGA TAT AAC CGG ATT CAA CAT CGG CAA TGT CGC
 R R S P E N S G R R Y N R I Q H G Q C A>

440 450 460 470 480 490
 TAC ACT TTC ATT CTT CCA GAA CAC GAT GGC AAC TGT CGT GAG AGT ACG ACA GAC CAG TAC
 Y T F I L P E H D G N C R E S T T D Q Y>

500 510 520 530 540 550
 AAC ACA AAC CCT CTG CAG AGA GAT GCT CCA CAC GTG GAA CGG GAT TTC TCT TCC CAG AAA
 N T N A L Q R D A P H V E P D F S S C K>

560 570 580 590 600 610
 CTT CAA CAT CTG GAA CAT CTG ATG GAA AAT TAT ACT CAG TGG CTG CAA AAA CTT GAG AAT
 L Q H L E H V M E N Y T O W L Q K L E N>

620 630 640 650 660 670
 TAC ATT GTG GAA AAC ATG AAG TCG GAG ATG GGC CAG ATA CAG CAG AAT GCA GTT CAG AAC
 Y I V E N M K S E H A Q I O Q N A V Q N>

680 690 700 710 720 730
 CAC ACG GCT ACC ATG CTG GAG ATA CGA ACC ACC CTC CTC TCT CAG ACT GCA GAG CAG ACC
 H T A T M L E I G T S L L S Q T A E Q T>

740 750 760 770 780 790
 AGA AAG CTG ACA GAT GTT GAG ACC CAG GTC CTA AAT CAA ACT TCT CGA CTT GAG ATA CAG
 R K L T D V E T Q V L N Q T S R L E I Q>

800 810 820 830 840 850
 CTG CTG GAG AAT TCA TTA TCC ACC TAC AAG CTA GAG AAG CAA CTT CTT CAA CAG ACA AAT
 L L E N S L S T Y K L E K Q L L Q Q T N>

860 870 880 890 900 910
 GAA ATC TTG AAG ATC CAT GAA AAA AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA ATG GAA
 E I L K I H E K N S L L E H K I L E M E>

920 930 940 950 960 970
 GGA AAA CAC AAG GAA GAG TTG GAC ACC TTA AAG GAA GAG AAA GAG AAC CTT CAA CGC TTG
 G K H K E E L D T L K E E K E N L Q G L>

980 990 1000 1010 1020 1030
 GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTG GAA AAG CAA TTA AAC AGA CCT ACC ACC
 V T R Q T Y I I Q E L E K Q L N R A T T>

1040 1050 1060 1070 1080 1090
 AAC AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG ATG GAC ACA GTC CAC AAC CTT GTC
 N N S V L O K Q Q L E L H D T V H N L V>

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Fig.4. (Cont.)

1100	1110	1120	1130	1140	1150
AAT CTT TCC ACT AAA GAA GGT GTT TTA CTA AAG GGA GGA AAA AGA GAG GAA GAG AAA CCA N L C T K E G V L L K G G K R E E E K P>					
1160	1170	1180	1190	1200	1210
TTT AGA GAC TGT GCA GAT GTC TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT F R D C A D V Y Q A C F N K S G I Y T I>					
1220	1230	1240	1250	1260	1270
TAT ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC AAT GGG GGA Y I N N H P E P K K V F C N H D V N G G>					
1280	1290	1300	1310	1320	1330
GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA AGT CTA GAT TTC CAA AGA GGC TGG AAG G W T V I Q H R E D G S L D F Q R G W K>					
1340	1350	1360	1370	1380	1390
GAA TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT E Y K M G F G N P S G E Y W L G N E F I>					
1400	1410	1420	1430	1440	1450
TTT CCC ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG F A I T S Q R Q Y M L R I E L H D W E G>					
1460	1470	1480	1490	1500	1510
AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC ATA GGA AAT GAA AAC CAA AAC TAT AGG N R A Y S Q Y D R F H I G N E K Q N Y R>					
1520	1530	1540	1550	1560	1570
TTC TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGG CTC ATC TTA CAC GGT L Y L K G H T G T A G K Q S S L I L H G>					
1580	1590	1600	1610	1620	1630
GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA TGT GCC CTC ATG A D F S T K D A D N D N C M C K C A L M>					
1640	1650	1660	1670	1680	1690
TTA ACA CGA CGA TGG TGG TTT GAT GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT L T G G W W F D A C G P S N L N G H F Y>					
1700	1710	1720	1730	1740	1750
ACT CGG CGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC T A G Q N H G K L N G I K W H Y F K G P>					
1760	1770	1780	1790	1800	1810
AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT TGA AAG CGCAATGT S Y S L R S T T H H I R P L D F>					
1820	1830	1840	1850	1860	1870
CAGAACCGATTATGAAAGCAACAAAGAAAATCGGAGAGCTGCCAGGTGAGAAACTCTTGTAACTTCTGAGCCGTTGAG					
1900	1910	1920	1930	1940	1950
ATATTTGCTCCCTTCCAGCAATAAGTGGTAGTTATGTGAAGTCACCAAGGTTCTTGACCCGTGAATCTGGAGCCGTTGAG					
1980	1990	2000	2010	2020	2030
TTCAACAGAGTCCTACTTGGGTGACAGTGCCTACGGTGGCTCGACTATAGAAAACCTCCACTGACTGTCGGCGTTAAA					
2060	2070	2080	2090	2100	2110
ACCGAAGAAACTGCTGAGCTTGTGCTTCAACTACTACTGACCTTATTTGCAACTA1GGTACCCAGATGATAAAAT					
2140					
ATGGTTAATTC					

Fig.5.

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10 20 30 40 50 60 70 80
 CAGCTGACTCAGGAGGCTCCATGCAACGCTCACACAGAGGAAACAAATCTCAGCTACTATGCATAAAATAC
 90 100 110 120 130 140 150 160
 TCAAGTTTAAAGAAGAAAACATTCATTGCACTGAAATAAATTTAAATTTAGAACAAAGCTAACAAATGCTAG
 170 180 190 200 210 220 230 240
 TTTCTATGATTCTCTTCAAAACGTTTCTTGAGGGGGAAAGCTCAACAAACAAAGCTTTACCTGAAATAAGAA
 250 260 270 280 290 300 310
 CTAGTTTAAAGGTCAAGAAGAAGGAGCAAGTTTGGAGAGCCACGGAAAGGAGTGTCTGGCACTACAAATGACA
 M T>
 320 330 340 350 360 370
 GTT TTC CTT TCC TTT GCT TTC CTC GCT GCC ATT CTG ACT CAC ATA CGG TGC AGC AAT CAG
 V F L S F A F L A A I L T H I G C S N Q>
 380 390 400 410 420 430
 CGC CGA AGT CCA GAA AAC AGT CGG AGA AGA TAT AAC CGG ATT CAA CAT CGG CAA TGT CGC
 P P S P E N S G R R Y N P I Q H G O C A>
 440 450 460 470 480 490
 TAC ACT TTC ATT CTT CCA GAA AAC GAT CGC AAC TGT CGT GAG AGT ACG ACA GAC CAG TAC
 Y T F I L P E H D G N C R E S T T D Q Y>
 500 510 520 530 540 550
 AAC ACA AAC GCT CTG CAG AGA GAT GCT CCA CAC GTG GAA CGG GAT TTC TCT TCC CAG AAA
 N T N A L Q R D A P H V E P D F S S Q K>
 560 570 580 590 600 610
 CTT CAA CAT CTG GAA CAT GTG ATG GAA ATT TAT ACT CAG TGG CTG CAA AAA CTT GAG AAT
 L Q H L E H V M E N Y T Q W L Q K L E N>
 620 630 640 650 660 670
 TAC ATT GTG GAA AAC ATG AAG TGG GAG ATG CGG CAG ATA CAG CAG AAT GCA GTT CAG AAC
 Y I V E N H K S E H A Q I Q O N A V Q N>
 680 690 700 710 720 730
 CAC ACG GCT ACC ATG CTG GAG ATA CGA ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC
 H T A T H L E I G T S L L S O T A E Q T>
 740 750 760 770 780 790
 AGA AAG CTG ACA CAT GTT GAG ACC CAG GAA CTA ATT CAA ACT TCT CGA CTT GAG ATA CAG
 R K L T D V E T Q V L H Q T S R L E I Q>
 800 810 820 830 840 850
 CTG CTG GAG ATT TCA TTA TCC ACC TAC AAG CTA GAG AAG CAA CTT CTT CAA CAG ACA ATT
 L L E N S L S T Y K L E K O L L O Q T N>
 860 870 880 890 900 910
 GAA ATC TTG AAG ATC CAT GAA AAA AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA ATC GAA
 E I L K I H E K N S L L E H K I L E M E>
 920 930 940 950 960 970
 CGA AAA CAC AAG GAA GAG TTG GAC ACC TTA AAG GAA GAG AAA GAG AAC CTT CAA CGC TTG
 G K H K E E L D T L K E E K E N L Q G L>
 980 990 1000 1010 1020 1030
 GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTC GAA AAG CAA TTA AAC AGA GCT ACC ACC
 V T R G T Y I I O E L E K O L N R A T T>
 1040 1050 1060 1070 1080 1090
 AAC AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG ATG GAC ACA GTC CAC AAC CTT GTC
 N N S V L Q K Q Q L E L H D T V H N L V>

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Fig.5. (Cont.)

1100 1110 1120 1130 1140 1150
 AAT CTT TGC ACT AAA GAA GTT TTA CTA AAG GGA GCA AAA AGA GAG GAA GAG AAA CCA TTT
 N L C T K E V L L K G G K R E E E K P F>
 1160 1170 1180 1190 1200 1210
 AGA GAC TGT GCA GAT GTC TAT CAA CCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT
 R D C A D V Y Q A G F N K S G I Y T I Y>
 1220 1230 1240 1250 1260 1270
 ATT AAT AAT ATG CCA GAA CCC AAA AAG CTG TTT TGC AAT ATG GAT GTC AAT GGG GGA CGT
 I N N M P E P K K V F C N H D V N G G G>
 1280 1290 1300 1310 1320 1330
 TCG ACT GTC ATA CAA CAT CTC GAA GAT GGA AGT CTC GAT TTC CAA AGA GGC TCG AAG GAA
 W T V I Q H R E D G S L D F O R G W K E>
 1340 1350 1360 1370 1380 1390
 TAT AAA ATG CGT TTT GCA AAT CCC TCC CGT GAA TAT TGC CTC CGG AAT GAG TTT ATT TTT
 Y K H G F G H P S G E Y W L C N E F I F>
 1400 1410 1420 1430 1440 1450
 GGC ATT ACC AGT CAG AGG CAG TAC ATG CTC AGA ATT GAG TTA ATG GAC TCG GAA GGG AAG
 A I T S Q R Q Y H L R I E L H D W E G N
 1460 1470 1480 1490 1500 1510
 CGA GGC TAT TCA CAG TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG
 R A Y S Q Y D P F H I G H E K Q N Y R L>
 1520 1530 1540 1550 1560 1570
 TAT TTA AAA CGT CAC ACT GGG ACA GCA GCA AAA CAG ACC AGC CTC ATC TTA CAC CGT CGT
 Y L K G H T G T A G K Q S S L I L H G A>
 1580 1590 1600 1610 1620 1630
 GAT TTC ACC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TCC AAA TGT GGC CTC ATG TTA
 D F S T K D A D N D N C H C K C A L H L>
 1640 1650 1660 1670 1680 1690
 ACA GGA GGA TGG TGG TTT GAT GCT TGT GGC CCC TCC AAT CTC AAT GGA ATG TTC TAT ACT
 T G G K W F D A C C G P S N L N G H F Y T>
 1700 1710 1720 1730 1740 1750
 GCG GGA CAA AAC CAT CGA AAA CTC AAT CGG ATA AAG TCG CAC TAC TTC AAA CGG CGG ACT
 A G Q N H G K L N G I K W H Y F K G P S>
 1760 1770 1780 1790 1800 1810
 TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT TCA AACCCAAATGTCAGAA
 Y S L R S T T H M I R P L D F>
 1820 1830 1840 1850 1860 1870 1880 1890
 CCCATTATGAAACCAACAAAGAAATCCGGAGAACCTGCCAGGTGAGAAACTGTTGAAACTTCAGAAGGAAACATATT
 1900 1910 1920 1930 1940 1950 1960 1970
 CTCCTCCCTCCACCAATAACTCTACTTATGAGTCACCAAGGTTCTGACCCGCTGAAATCTGGACCGCTTCACT
 1980 1990 2000 2010 2020 2030 2040 2050
 AACACTCTACTTGGGTGACAGTCTCACCTGCGACTATAGAAAATCCACTGACTCTCGGCTTTAAACCGA
 2060 2070 2080 2090 2100 2110 2120 2130
 AGAAACTGCTGAGCTTCTCTCTCAAAACTACTACTCGACCTTATTTGCAACTATGAGCCACATGATAAAATATG
 2140
 TAATTT

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Fig.6.

10	20	30	40	50	60	70	80
GAATTCTCGGTTGGTGTATCTCTCCAGCCTTGAGGGAGGGAAACAACACTGTAGGATCTGGGAGAGAGGAACAAA							
90	100	110	120	130	140	150	160
GGACCGTGAAAGCTGCTCTGTAAGCTGACACAGCCCTCCAAAGTGAGCAGGACTGTCTTCCACTGCAATCTGACAG							
170	180	190	200	210	220	230	240
TTTACTGCATGCCCTGGAGAGAACACAGCAGTAAACCCAGGTTGCTACTCGAAAAAGAGGAAAGAGAACACTTTCATTG							
250	260	270	280	290	300	310	320
ACGGACCCAGCCATGGCAGCGTAGCACCCCTGGCTTCAGACGGCAGCAGCTGGGACTCTGGACGTGTGTTGCCCTCA							
330	340	350	360	370	380		
AGTTTGCTAACGCTGCTGTTTATTACTGAAGAAAAGA ATG TGG CAG ATT GTT TTC TTT ACT CTG AGC TGT							
			H W Q I V F F T L S C >				
390	400	410	420	430	440		
GAT CTT GTC TTG GCC GCA GCC TAT AAC AAC TTT CCG AAG AGC ATG GAC AGC ATA GGA AAG							
D L V L A A A Y N N F P K S M D S I G M							
450	460	470	480	490	500		
AAG CAA TAT CAG CTC CAG CAT CGG TCC TCC AGC TAC ACT TTC CTC CTG CCA GAG ATG GAC							
K Q Y Q V Q H G S C S Y T F L L P E M L							
510	520	530	540	550	560		
AAC TCC CGC TCT TCC AGC CCC TAC GTG TCC AAT GCT GTG CAG AGG GAC GCG CGG CTC							
N C F S S S S P Y V S N A V Q R D A I L							
570	580	590	600	610	620		
GAA TAC GAT GAC TCG GTG CAG AGG CTG CAA GTG CTG GAG AAC ATC ATG GAA AAC AAC ACT							
E Y D D S V Q R L Q V L E N I M E N N T							
630	640	650	660	670	680		
CAG TGG CTA ATG AAG CTT GAG AAT TAT ATC CAG GAC AAC ATG AAG AAA GAA ATG GTA GAG							
Q W L M K L E N Y I Q D N M K K E M V E							
690	700	710	720	730	740		
ATA CAG CAG AAT GCA GTC CAG AAC CAG AGC GCT GTG ATG ATA GAA ATA CGG ACA AAC CTG							
I Q Q N A V Q N Q T A V H I E I G T N L >							
750	760	770	780	790	800		
TTG AAC CAA ACA GCT GAG CAA ACC CGG AAG TTA ACT GAT GTG GAA GCC CAA GTC TTA AAT							
L N O T A E Q T R K L T D V E A Q V L N >							
810	820	830	840	850	860		
CAG ACC ACG AGA CTT GAA CTT CAG CTC TTG GAA CAC TCC CTC TCG ACA AAC AAA TTG GAA							
Q T T R L E L Q L L E H S I S T N K L E							
870	880	890	900	910	920		
AAA CAG ATT TTG GAC CAG ACC ACT GAA ATA AAC AAA TTG CAA GAT AAG AAC ACT TTC CTA							
K C I L D O T S E I N K L Q D K N S F L							
930	940	950	960	970	980		
GAA AAG AAG GTG CTA GCT ATG GAA GAC AAG CAC ATC ATC CAA CTA CAG TCA ATA AAA GAA							
E K K V L A M E D K H I I Q L Q S ! K E							
990	1000	1010	1020	1030	1040		
GAG AAA GAT CAG CTA CAG GTG TTA GTC AAG CAA ATT TCC ATC ATT GAA GAA CTA GAA							
E K D Q I O V L V S K C N S I I E E L E							
1050	1060	1070	1080	1090	1100		
AAA AAA ATA GTG ACT GCC ACG GTG AAT AAT TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC							
K K I V T A T V N N S V L Q K Q Q H D L							

Fig.6. (Cont.)

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1110 1120 1130 1140 1150 1160
 ATG GAG ACA GTT AAT AAC TTA CTG ACT ATG ATG TCC ACA TCA AAC TCA GCT AAG GAC CCC
 M E T V N N L L T M H S T S N S A K D P>
 1170 1180 1190 1200 1210 1220
 ACT GTT GCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCT GAA GTA TTC AAA TCA CGA
 T V A K E E Q I S F R D C A E V F X S G>
 1230 1240 1250 1260 1270 1280
 CAC ACC ACA AAT GCC ATC TAC ACC TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC
 H T T N G I Y T L T F P N S T E E I K A>
 1290 1300 1310 1320 1330 1340
 TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GCC
 Y C D M E A G G G G W T I I Q R R E D G>
 1350 1360 1370 1380 1390 1400
 AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT TCA CGA
 S V D F Q R T W K E Y K V G F G N P S G>
 1410 1420 1430 1440 1450 1460
 GAA TAT TGG CTG CGA AAT GAG TTT TGT TCG CAA CTG ACT AAT CAG CAA CGC TAT CTG CTT
 E Y W L G N E F V S Q L T N Q O R Y V L>
 1470 1480 1490 1500 1510 1520
 AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA CAT TTC TAT
 K I H L K D W E G N E A Y S L Y E H F Y>
 1530 1540 1550 1560 1570 1580
 CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GCC CGC
 L S S E E L N Y R I H L K G L T G T A G>
 1590 1600 1610 1620 1630 1640
 AAA ATA ACC AGC ATC AGC CAA CCA CGA AAT GAT TTT AGC ACA AAG GAT CGA GAC AAC GAC
 K I S S I S O P G N D F S T K D G D N D>
 1650 1660 1670 1680 1690 1700
 AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA CGA GGC TGG TGG TTT GAT GCA TGT CGT
 K C I C K C S G M L T G G W W F D A C G>
 1710 1720 1730 1740 1750 1760
 CCT TCC AAC TTG AAC CGA ATG TAC TAT CCA CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC
 P S N L N G M Y Y P Q R Q N T N K F N G>
 1770 1780 1790 1800 1810 1820
 ATT AAA TGG TAC TAC TGG AAA CGC TCA CGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC
 I K W Y Y W K G S G Y S L K A T T M M I>
 1830 1840 1850 1860 1870 1880 1890 1900
 CGA CCA CGA GAT TTC TAAACATCCCAGTCCACCTGAGGAATGTCCTCGAATCTTTCAAAAGACTTAAGCCCACT
 R P A D F>
 1910 1920 1930 1940 1950 1960 1970 1980
 CGACTGAAAGTCACGGCTGGCACTGTGTCCTCTTCCACACAGAGGGCGTGTGCTCGGTGCTGACGGGACCCACATGTT
 1990 2000 2010 2020 2030 2040 2050 2060
 CCAGATTAGAGCTGTAACCTTATCACTTAAACTTGCATCACTTAAACGGACCAAGCAAGACCCATAACATCCATAATT
 2070 2080 2090 2100 2110 2120 2130 2140
 GTGATTAGACAGAACACCTATGCAAAAGATGAACCCGAGGCTGAGAATCAGACTGACAGTTACAGACGGCTGTCACAA
 2150 2160 2170 2180 2190 2200 2210 2220
 CCAAGAAATGTTATGTCGAAGTTATCAGTAATAACTGAAAGAGAGACCCATAACATCCATAATT
 2230 2240 2250 2260 2270 2280
 ACTCCATTCTGAGGACTGTTATACACTGTCATAACCCATAATGTCCTGAAATT

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Fig.7.

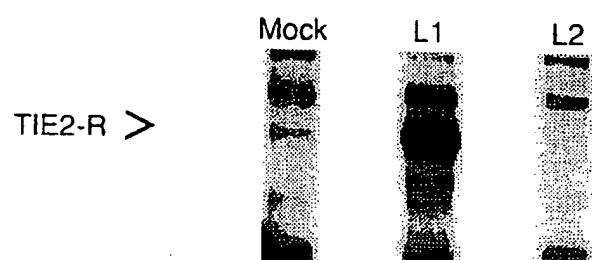
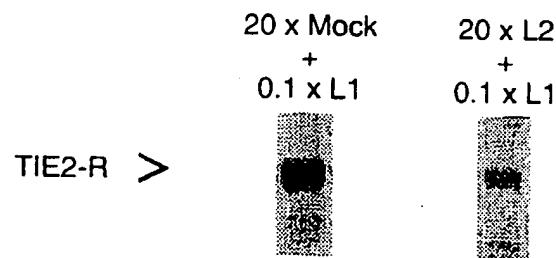
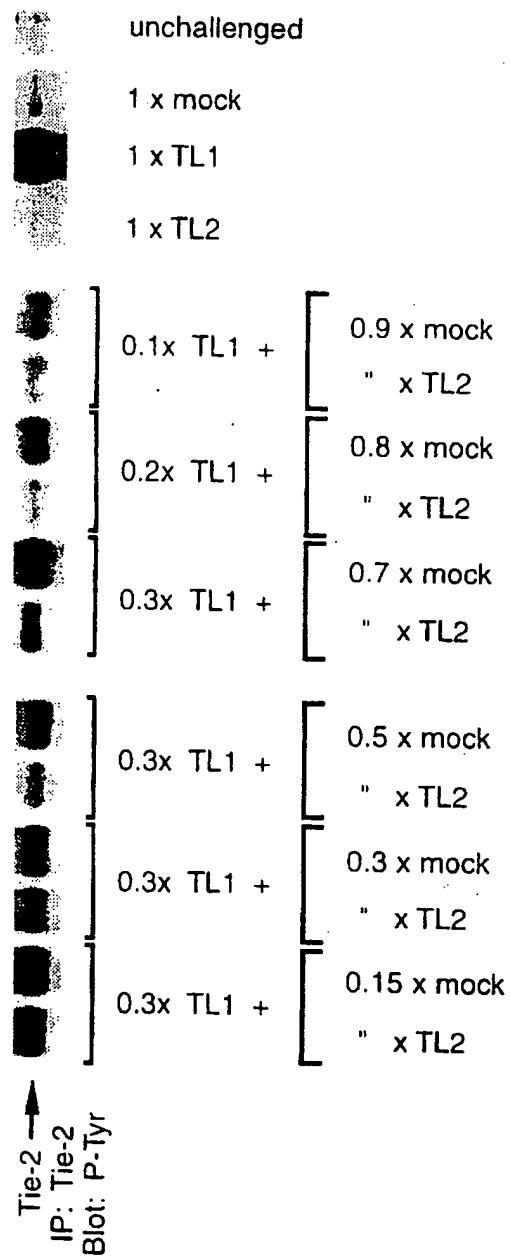


Fig.8.



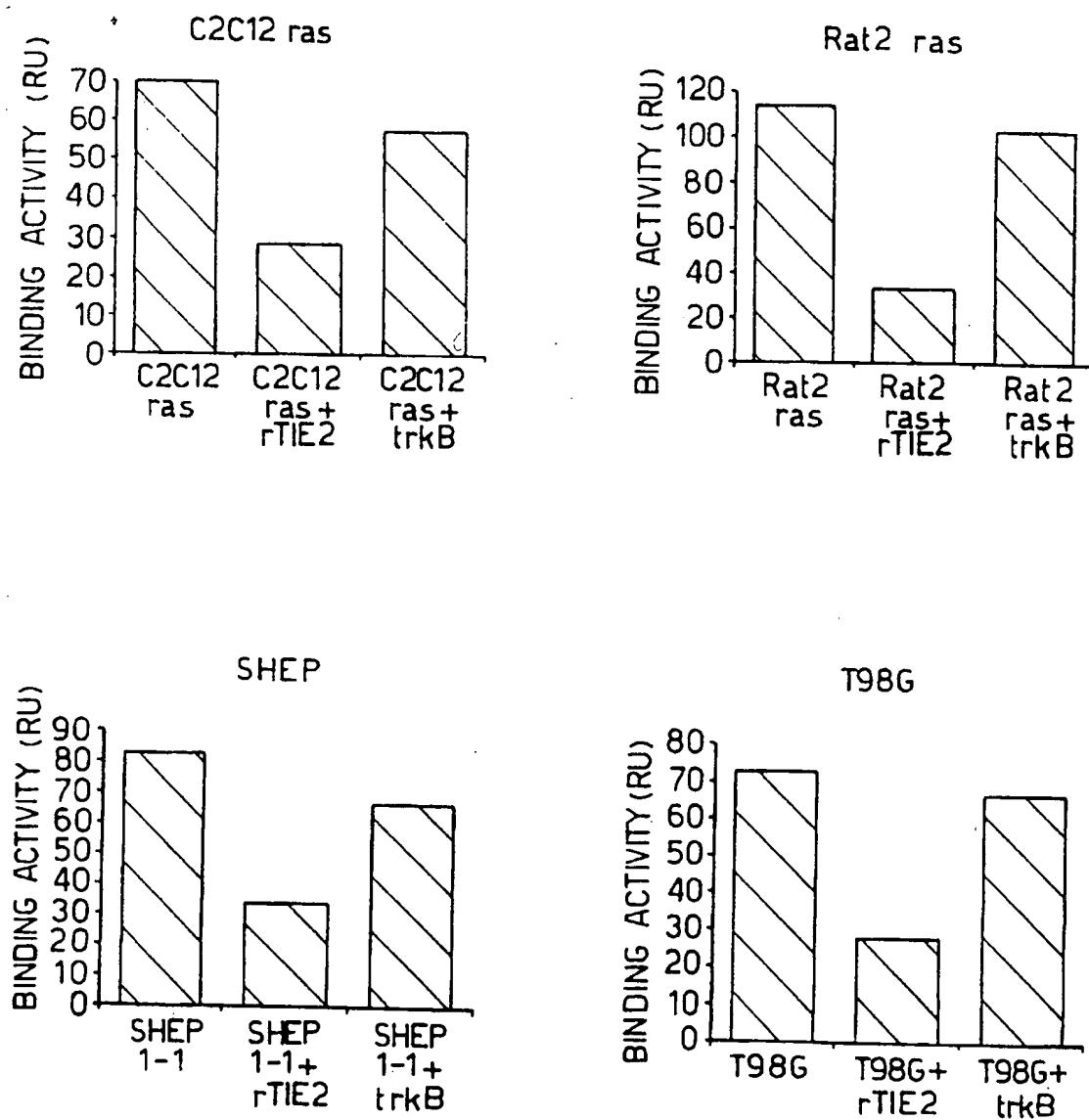
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Fig.9.



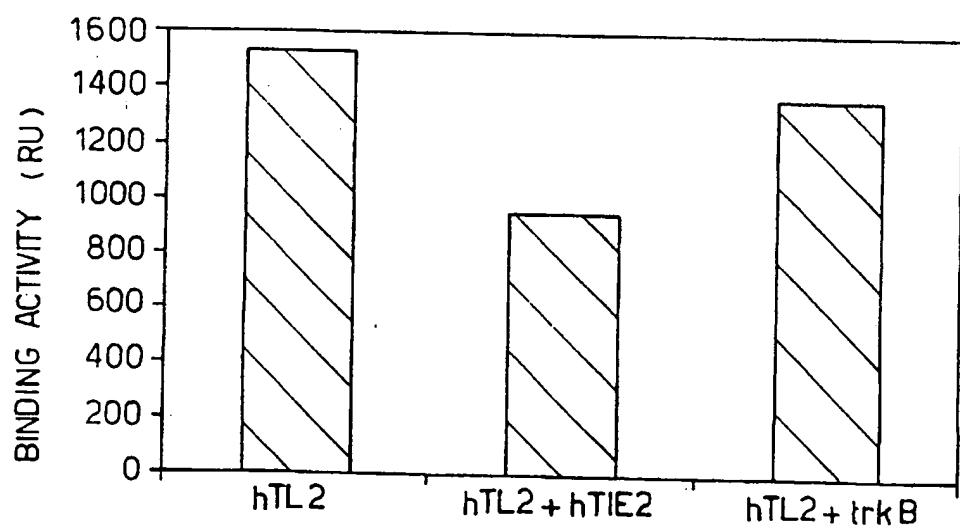
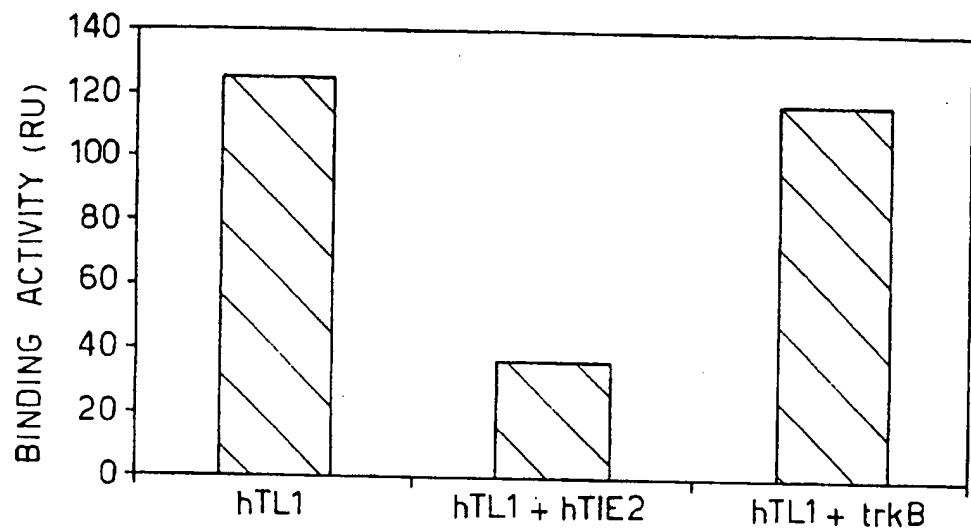
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Fig.10.



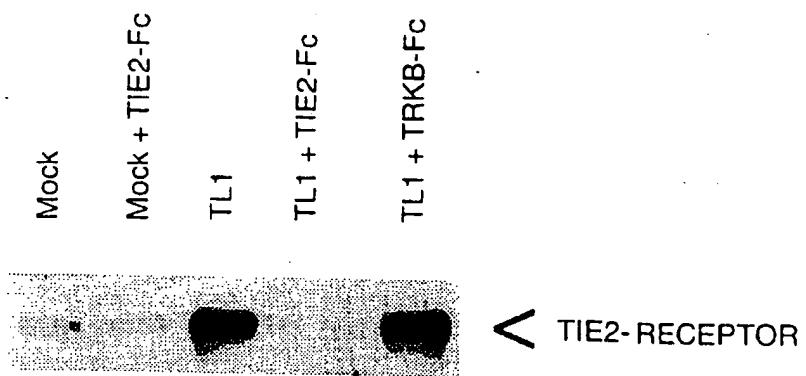
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Fig.11.



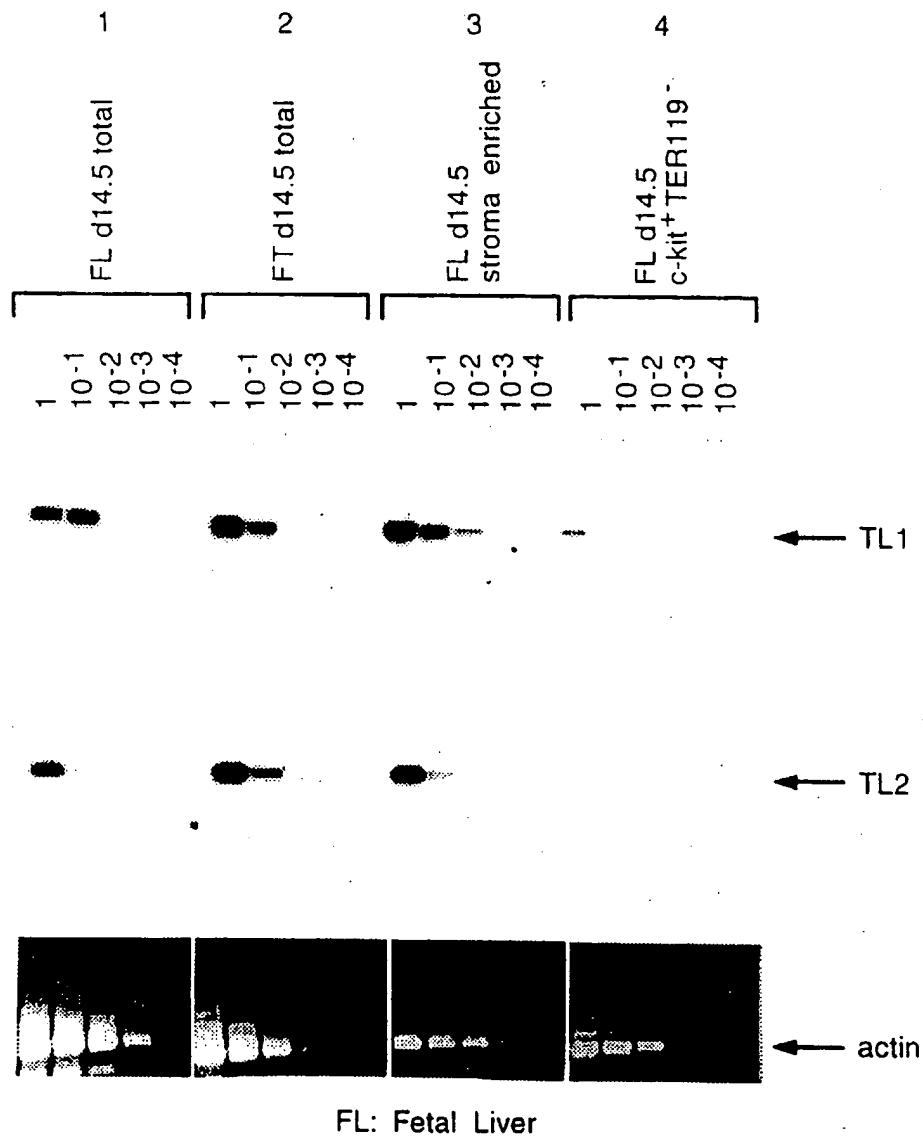
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Fig.12.



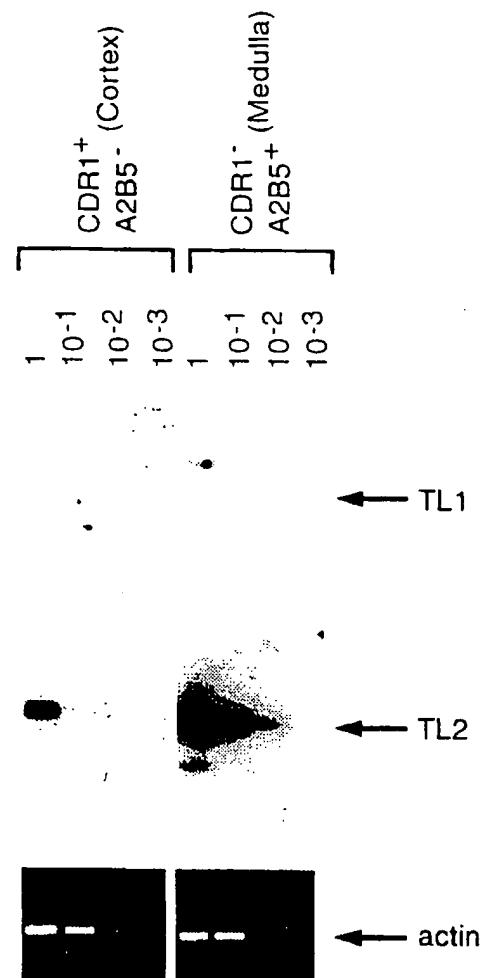
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Fig.13.



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Fig.14.

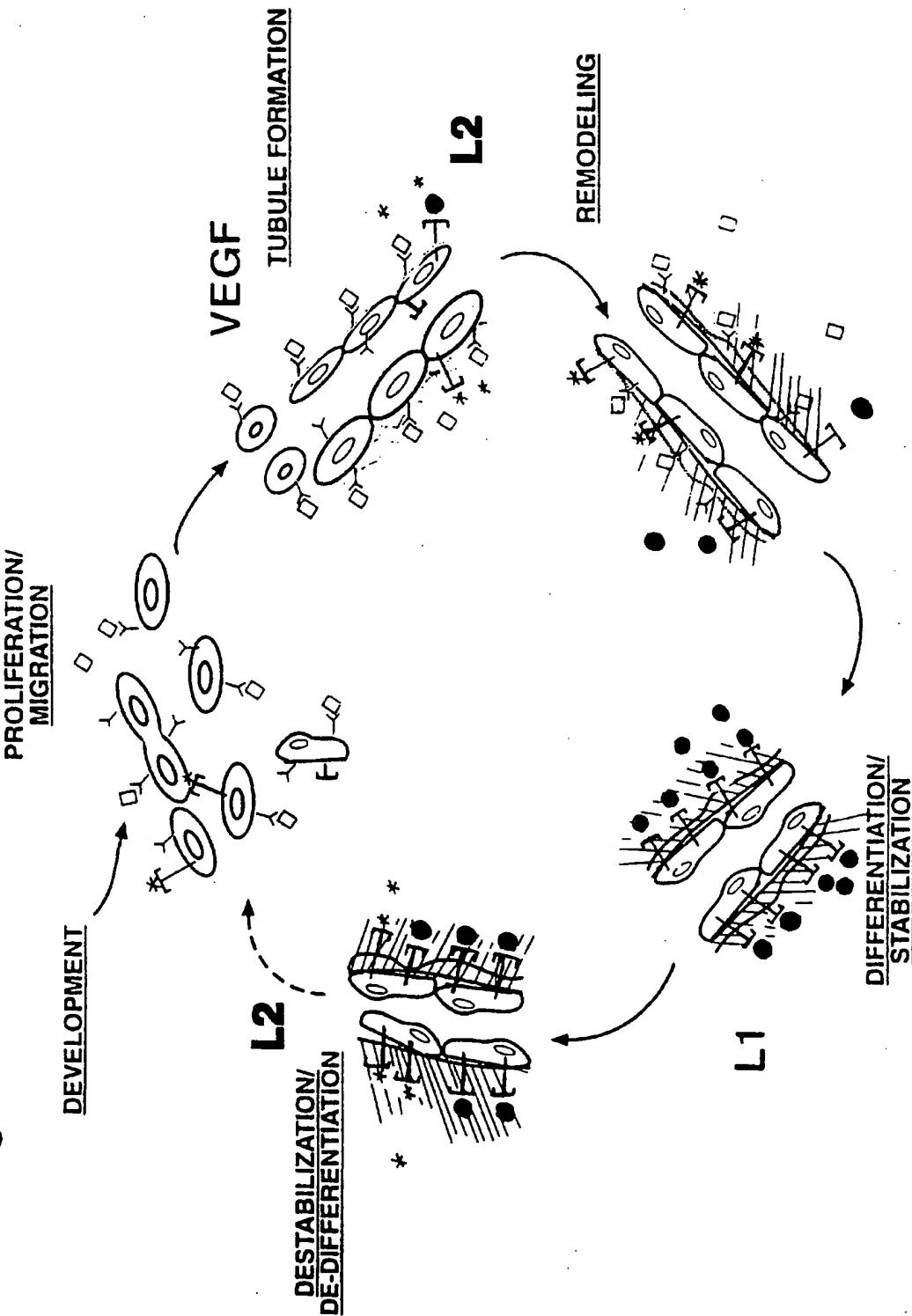


Fetal Thymus E17.5

CDR1⁺ : Cortical stromal cellsA2B5⁺ : Medulla stromal cells

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Fig. 15. ANGIOGENESIS



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Fig.16.

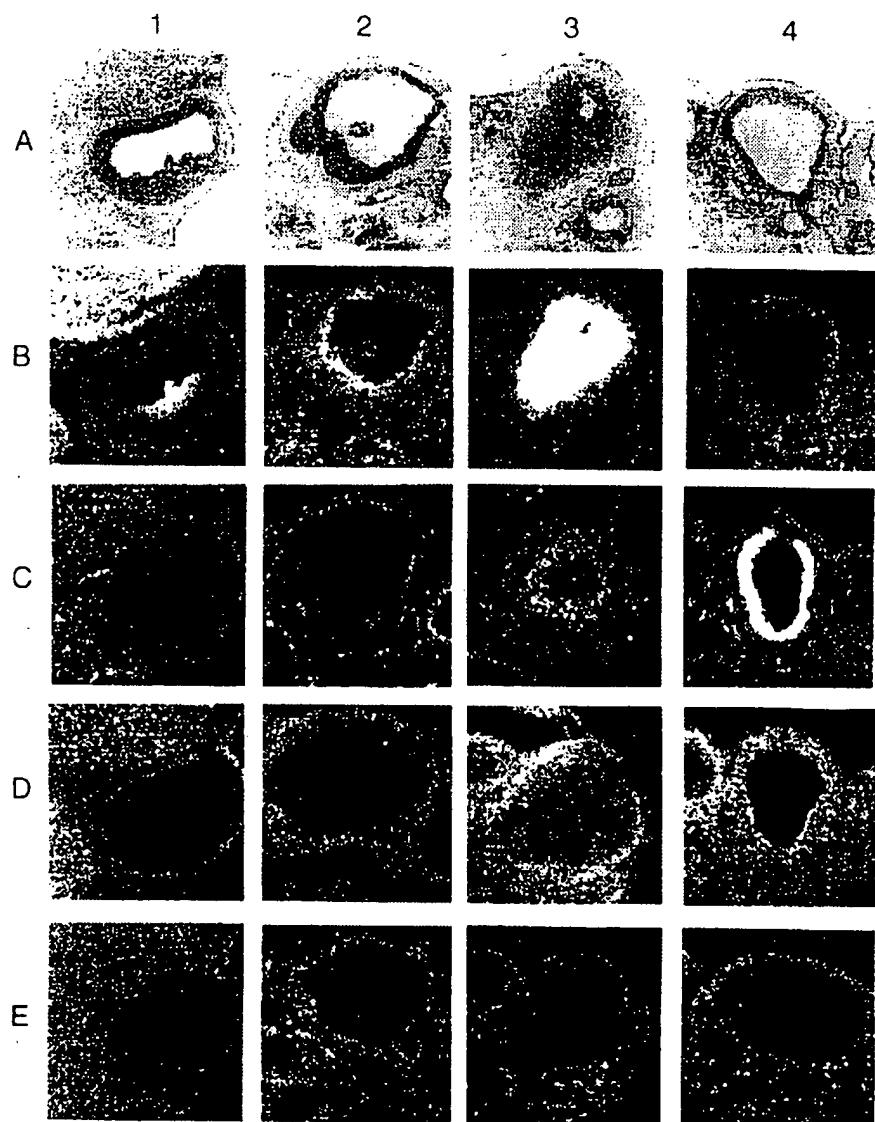


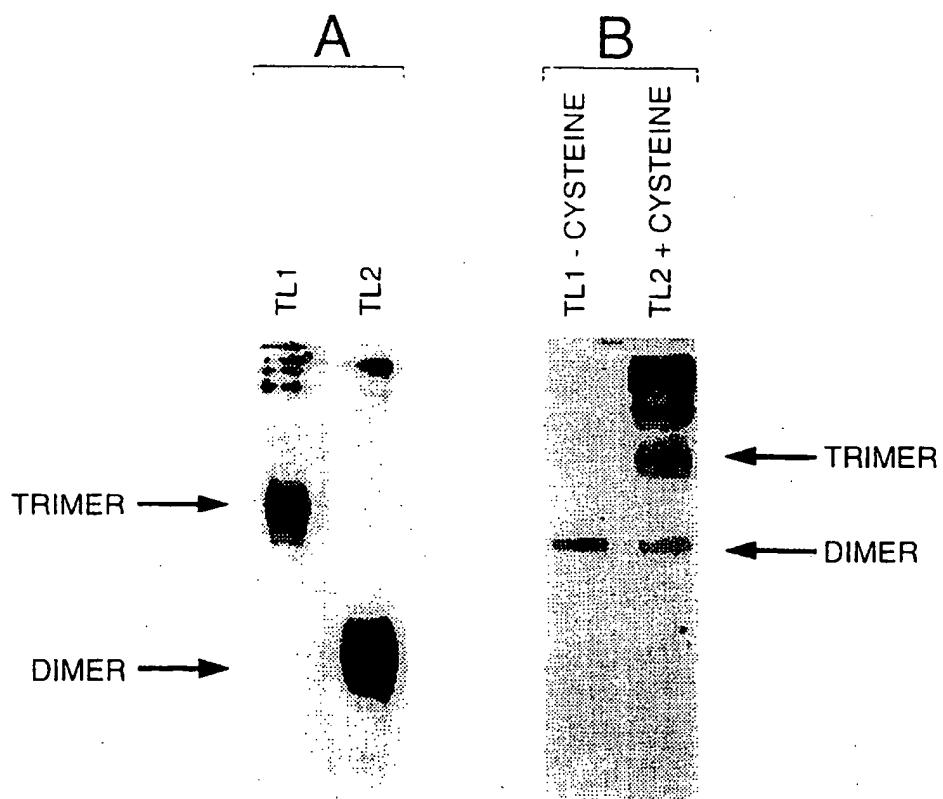
Fig. 17.

		10	20	30	40	50	60	70	80	
TL1		NQRSPENSG RRYNRJQHQ CAYTFILPEH DGNCRESTTD QINTVALQD APHEPDESS QKUQHLEHM ENYQWQLQK								
TL2		2257ANFRkSndisig kkgygVQHGs CsYTFILP2m Dn-CR3-sss	10	20	30	40	50	60	70	80
		pxysNAVQRD API-EJdssv QrLQvAENIM ENtQWLMK								
TL1		90	100	110	120	130	140	150	160	
		ENTIVENNS EMQIOQNAV QNETPATMELI GTSLLSQPAE QTRKLTDVET QVNLNTSRLE IOLLENSTY YKLERQILQQ								
TL2		90	100	110	120	130	140	150	160	
		ENTIQdMNEK EnveiQQNAV QNGTAVMELI GTRLLQPAE QTRKLTDEA QVNLNTSRLE IOLLENSTY YKLERQILQQ								
TL1		170	180	190	200	210	220	230	240	
		TNEILKTHEK NSTELEKVL MEDRKH1qkq sixKEERdQQLQ VIVskQnsII eELExLvtA TtNtSVLQKQ QhdLMetVtN								
TL2		170	180	190	200	210	220	230	240	
		TSEmH1qkq NSTELEKVL MEDRKH1qkq sixKEERdQQLQ VIVskQnsII eELExLvtA TtNtSVLQKQ QhdLMetVtN								
TL1		250	260	270	280	290	300	310	320	
		LYNL-CtREGV LLRGGRREEE KPFRCADVV QAGFNKSGY TIVNNNTEP KKYFCNMVDN CGGWTVIQHR EDGSLDFQG								
TL2		250	260	270	280	290	300	310	320	
		L1 emtstensa kdpvtvaaEEq 1sFRDCAeEfV ksgHttmGtV TltfpNstEe ikayCdMeag CGGWTIIQFR EDGSLDFQrt								
TL1		330	340	350	360	370	380	390	400	
		WREYXMGFGN PSGETMLGNE FIRATISQD YMRLIEDN EGNRAYSQD RFHGNENQN YRLYLGHTG TAGQSSLIL								
TL2		330	340	350	360	370	380	390	400	
		WREYXMGFGN PSGETMLGNE FysqtlmQr YvLkLkLk EGNEAyslye hysLsEEIN YRHLkLkLk TAGLSSisq								
TL1		410	420	430	440	450	460	470		
		HGADFSTRDA DNDNCMCKA LMUTGQWFD ACGPSNLGM FTAGQHNGK LNGTKWHTK GPSYSLRSTT MMTRPLDF								
TL2		410	420	430	440	450	460	470	480	
		pmDFSTRDg DNDkCICRCS qMILGQWFD ACGPSNLGM yrpqCQNTk ENGKXWYK GsgXSTkatt MMTRPdF								

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Fig.18.

COVALENT MULTIMERIC STRUCTURE OF
TL1 AND TL2 AND THEIR INTERCONVERSION
BY THE MUTATION OF ONE CYSTEINE



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Fig.19.

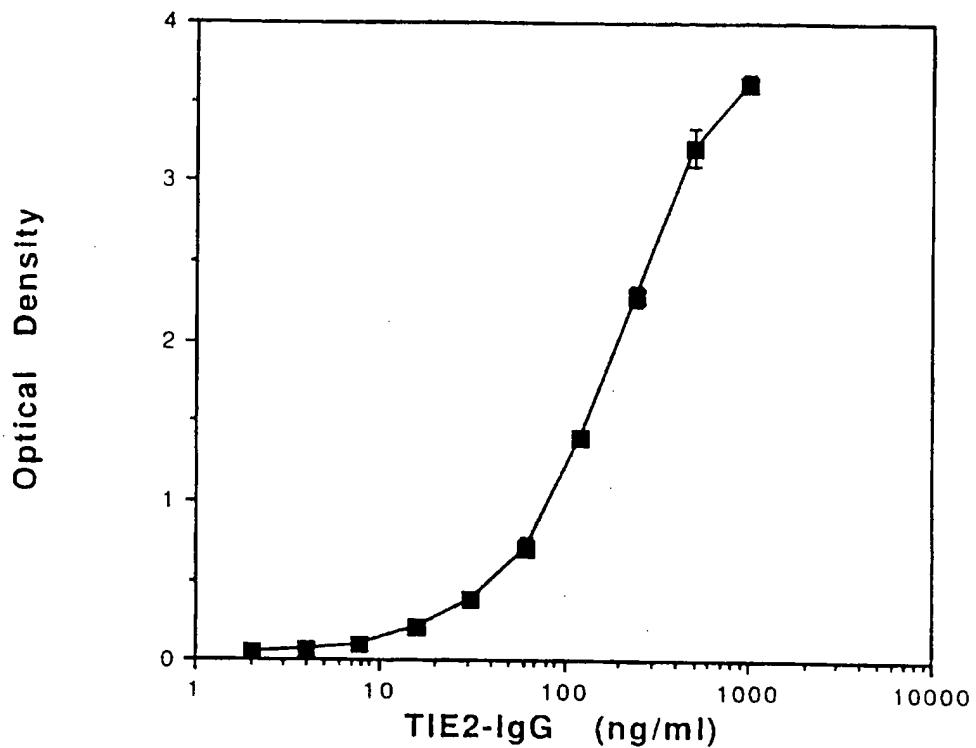
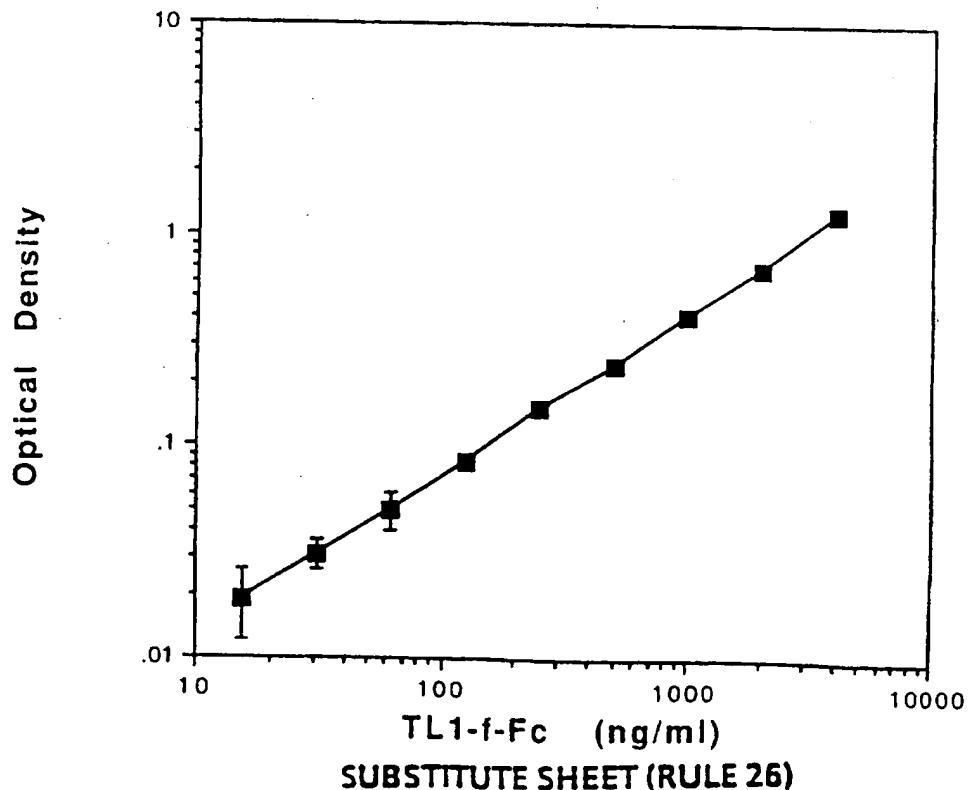


Fig.20.



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Fig.21.

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Fig.21(Cont i).

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770	*	*	780	*	*	790	*	*	800	*	*	810	*	*	820	*	*	830	*	*	840	*		
T	C	T	L	A	N	L	K	H	N	L	A	L	S	S	S	S	L	Q	Q	Q	Q	L>		
850	*	*	860	*	*	870	*	*	880	*	*	890	*	*	900	*	*	910	*	*	920	*		
T	E	F	V	Q	R	L	V	R	I	V	A	Q	D	Q	H	P	V	S	L	K	T	P	V	F
940	*	*	950	*	*	960	*	*	970	*	*	980	*	*	990	*	*	1000	*	*	1010	*		
D	C	A	E	I	K	R	S	G	V	N	T	S	G	V	Y	T	I	Y	E	T	N	H	T	K
1020	*	*	1030	*	*	1040	*	*	1050	*	*	1060	*	*	1070	*	*	1080	*	*	1090	*		
V	F	C	D	H	E	T	D	G	G	W	T	L	I	Q	H	R	E	D	G	S	V	H	F	Q
1100	*	*	1110	*	*	1120	*	*	1130	*	*	1140	*	*	1150	*	*	1160	*	*	1170	*		
W	E	E	Y	K	E	G	F	G	H	V	A	R	E	H	W	L	G	H	E	A	V	H	R	L
1190	*	*	1200	*	*	1210	*	*	1220	*	*	1230	*	*	1240	*	*	1250	*	*	1260	*		
T	A	Y	L	L	R	V	E	L	W	D	H	E	G	R	Q	T	S	I	Q	Y	E	H	F	Q
1270	*	*	1280	*	*	1290	*	*	1300	*	*	1310	*	*	1320	*	*	1330	*	*	1340	*		
E	R	Q	R	Y	S	L	V	H	D	S	S	S	A	G	R	K	R	S	L	A	P	Q	G	T
1360	*	*	1370	*	*	1380	*	*	1390	*	*	1400	*	*	1410	*	*	1420	*	*	1430	*		
F	S	T	K	D	H	C	A	K	C	A	Q	H	L	S	G	G	W	G	F	D	A	C>		

Fig.21(Cont ii).

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1440	*	1450	*	1460	*	1470	*	1480	*	1490	*	1500	*	1510	*
GGC CTC TCC AAC CTC ATT GGC ATC TAC TAT TCA GTC CAT CAG CAC TTG CAC ARG ATC ATT GGC ATC CGC TGG CAC TAC TTC CGA															
G L S H L N G I Y Y S V H Q H L K I N G I R H Y F R															
1530	*	1540	*	1550	*	1560	*	1570	*	1580	*	1590	*	1600	*
GGC CCC AGC TAC TCA CTG CAC CGC ACA CGC ATG ATG CTG RGG CCA ATG GGT GCC ATG TGA CACA CAGCCCTGCA GAGACTGATG															
G P S Y S L H G T R H H L R P H G A *															
1610	*	1620	*	1630	*	1640	*	1650	*	1660	*	1670	*	1680	*
CCGCTGGGG ATTCTCAACC CAGGTGACTC TGTGCAAGCT GGGCCCTGCT CAGRAATCAG TGGCCAGGGC TCATCTTGAC ATTCTGGAAC ATCGGAACCA															
1710	*	1720	*	1730	*	1740	*	1750	*	1760	*	1770	*	1780	*
GCTTACCTTG CCCCTGAAATT ACAGAATTC ACCTGCCCTCC CTGTTGCCCTT CTAAATTGTA ATTTCGCTGGG TGCTTGAGG CACCTGCCCTC TGTTGGACCC															
1810	*	1820	*	1830	*	1840	*	*	*	*	*	*	*	*	*
ATRACTCTTC CCCCTCTGTC TGCTTGCCCC GGAAATCCCTG CCATGAACCT															

Fig.22.

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10 20 30 40 50 60 70 80
 MLDGLLJLA THAAQHHRGP EAGGHRQIHQ VRRGQCSYTF VVPEPDICQL APTAPEALG GSNSLQRDLP ASRLHLTDWR
 hTl1. af.aa.i.thi -gcsn.r.s. .ns.r-rynr igh...a... il..h-dg-n crestdq-y nt.a...a- ---p.-v--e>
 chTl1. af.aa..ahi -gctt.r... .s.r-ffnr igh...t... il..q-dg-n crestdq-y nt.a...a- ---p.-v--e>
 mTl1. mcvflsaffaaillthigcsn.r.n. n..r.-yxr igh...a... il..h-gn- cres.t.qy- nt.a...a. -----v-e-->
 mTl2. mwqififefgwd.v.. saysnfrksv dst.r..y. .qn.p..... ll..t.s.r- -ssss.-ym- -av...a. ---.dy-.->
 hTl2. mwqivfflscd.v.. aaynfrksm dsi.kk..y. .qh.s..... ll..m.n.r- -ssss.-yv- -av...a. ---.ey-.->

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90 100 110 120 130 140 150 160
 AORAQRAORV SOLEKILENN TQKULXKLEQS IKUNLRSHV QAOQDTIQNQ STTMALGAN LMNQTKAQTH KLTAVEAQVL
 pdf--ss.k1 qh..hvm..yq...ny .ve.mk.ema .i..nav..h .a...e..r .1..ae..rd..t...>
 qdf--sf.k1 qb..hvm..yq...sy .ve.mk.em. .1..nav..h .a...e..r .1..ae..rd..t...>
 pdfs--s.k1 qh..hvm..yq...ny .ve.mk.ema .i..nav..h .a...e..r .1..ae..rd..t...>
 -dsv..1..-n...m...n.m...m...n.m...m...d...>
 -dsv..1..-n.m...m...n.m...m...n.m...m...d...>

170 180 190 200 210 220 230 240
NOJLHMKTQM LENSLSTNKL EPMQMLMOSRE LQRLQGRNRA LETRLQALEA QHQAOQLNSLQ EKREQLHSLL GHQQTGLANL
 hTl1. ...srlei.1y... .k.1.q.tn. ilkinek.s1 .hkilem.g k.kee.dt.k .ek.n.qg.v tr..yiqe.>
 chTl1. ...srlei.1y... .k.1.q.tn. ilkinek.s1 .hkilem.e r.keeemd.t.k .ek.n.q..v tr..syiqe.>
 mTl1. ...srlei.1y... .k.1.q.tn. ilkinek.s1 .hkilem.g k.kee.dt.k .ek.n.qg.v sr..fiiqe.>
 mTl2. ...trlel.1 .qh.i..... .k.i.d.ts. ink..nk.sf .qkvldm.g k.se..q.mk .qkde.qv.v sk..svide.>
 hTl2. ...trlel.1 .h..... .k.i.d.ts. ink..dk.sf .kkvl.m.d k.ii..q.ik .ekd..qv.v sk..nsiie.>

250 260 270 280 290 300 310 320
 KHNHALSN SSSLQQQQQ LTFVQLVR IV---AQ-DQHP---V--S L-XTPXPYFQD CAEIKRSGVN TSGVYTIYET NMTKPLKVF
 hTl1. ekq.nrat. n.v..k..le .ndt.bn. n 1c---tkevllk--g--g k-reeekp.r. ..dvyqa.f. k..i....in ..pe.k....>
 chTl1. ekq.nkact. n.v..k..le .ndt.bn..it 1c---sk-egvllkn--a k-.eeekp.r. ..dvyq..f. k...in .vsd.k....>
 mTl1. ekq.sratn. n.i..k..le .ndt.bn..s 1c---tk-egvll--lkgg k-reeekp.r. ..dvyqa.f. k..i....fn ..pe.k....>
 mTl2. ekk.vcatv. n.l..k..hd .m.t.ns.lt mss-pn-skss--.aa ir.eeqtt.r. ...fk..lt ..i..ltfp .s.eei/ay.>
 hTl2. ekkivtav. n.v..k..hd .m.t.mn.lt msssns-akd.--t--v a..eeqis.r. ...yfk..ht .n.i..ltfp .s.eei/ay.>

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Fig.22(Cont).

mTL3	330	340	350	360	370	380	390	400
hTL1.	DME T EGGNT LIQHREDGSV NFORTWEEYK E G FGNYAREH WLGNE AVHRL TSR TAY LLRV ELHDWEG RQT SIQYENFQLG							
chTL1.	n.dvn.....	v.....1	d...g.k...	m....psg.yfifai	.qrq.m..i	..m....nra	ys..dr.hi..>
mTL1.	n..vn....	v.....1	d..xg.k...	m...spsg..fifai	.qrq.s..i	..m....nra	ys..dr.hi..>
mTL2.	n.dvn....	v.....1	d...g.k...	m....psg.yfifai	.qrq.m..i	..m....nra	ys..dr.hi..>
hTL2.	..dvg....	v.....1	d...g.k...psg.yf.sq.	.gqhr.v.ki	q.k....nea	hs1.dh.y.a>
	...ag....	i..x.....	d....k...	v....psg.yf.sq.	.nqqr.v.ki	h.k....nea	ys1..h.y.s>
mTL3	410	420	430	440	450	460	470	480
hTL1.	SERQRYSLV NDSSSSAGRK NSLAPQGTXF STKDHDNDNC MCKCAQMLSG GWWFDACGLS NLNG YYSVH QH U HKINGIR							
chTL1.	n.k..n.r.y1	kghtgt..	kq s..ilh.ad.a.....1..t.p.mf.tagk>
mTL1.	n.k..n.r.y1	kgh.gt..	kq s..ilh.ae.a.....1..t.p.mf..agk>
mTL2.	n.k..n.r.y1	kghtgt..	kq s..ilh.ad.a.....1..t.p.mf.tagk>
hTL2.	g.esn.rihl	tgltgt..	aki s.isqp.sd.s...k.	i...s.....p.q..pqkk>
	..eln.rihl	kgltgt..	ki s.isqp.nd.g...k.	i...s...t.p.m..pqrk>
mTL3	490	500						
hTL1.	WHYFRG PSYS I HGTRMMHLRP HGA*							
chTL1.k.....rs.t..i..	1df					
mTL1.k.....rs.t..i..	1df>					
mTL2.k.....rs.t..i..	1df>					
hTL2.y.wk.sq..ka.t..i..	adfs>					
v.wk.sq..ka.t..i..	adfs>					

Fig.23.

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ATG CTC TCC CAG CTA GCC	ATG CTG CAG GGC AGC CTC	CTT GTG GTT GCC ACC ATG TCT GTG GCT	50		
M L S Q D A M	Q L Q G S L	L V V A T M S V A	60		
70	80	90	100		
CAA CAG ACA AGG CAG GAG GCG GAT AGG GGC TGC GAG ACA CTT GTA GTC CAG CAC GGC CAC TGT AGC	Q T R Q E A D R G C E T L V V Q H G H C S	110	120		
140	150	160	170		
TAC ACC TTC TTG CTG CCC AAG TCT GAG CCC TGC CCT CCG GGG CCT GAG GTC TCC AGG GAC TCC AAC	Y T F L L P K S E P C P P G P E V S R D S N	180	190		
200	210	220	230		
AC CTC CAG AGA GAA TCA CTG GCC AAC CCA CTG CAC CTG GGG AAG TTG CCC ACC CAG CAG GTG AAA	T L Q R E S L A N P L H L G K L P T Q Q V K	240	250		
270	280	290	300		
CAG CTG GAG CAG GCA CTG CAG AAC ACG CAG TGG CTT AAG AAG CTA GAG AGG CTC ATC AAG AGC	Q L E Q A L Q N N T Q W L K K L E R A I K T	310	320		
340	350	360	370		
ATC TTG AGG TCG AAG CTG GAG CAG CAG GTC CAG CAG CAA ATG GCC CAG AAT CAG AGC CCC ATG CTA	I L R S K L E Q V Q Q M A Q N Q T A P M L	380	390		
400	410	420	430		
GAG CTG GCC ACC AGC CTC CTG AAC CAG ACC ACT GCC CAG ATC CGC AAG CTG ACC GAC ATG GAG GCT	E L G T S L L N Q T T A Q I R K L T D M E A	440	450		
470	480	490	500		
CAG CTC CTC AAC CAG ACA TCA AGA ATG GAT GCC CAG ATG CCA GAG ACC TTT CTG TCC ACC AAC AAG	Q L L N Q T S R M D A Q M P E T F L S T N K	510	520		
530	540	550	560		
CTG GAG AAC CAG CTG CTG CTA CAG AGG CAG CTC CAG CTC CAG CTC CAG CTC CAA AAC AGC GCG CTC	L E N Q L L Q R Q K L Q Q L Q G N S A L	570	580		
				590	

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Fig.23(Cont i).

GAG AAG CGG TTG CAG GCC CTC GAG ACC AAG CAG CAG GAG GAG CTC GCC AGC ATC CTC AGC AAG AAG	600	610	620	630	640	650	660
E K R L Q A L E T K Q Q E E L A S I L S K K							
GCG AAG CTG CTG AAC ACG CTC AGC CGC CGC AGC GCC CTC ACC AAC ATC GAG CGC CGC CTG CGC	670	680	690	700	710	720	
A K L L N T L S R Q S A A L T N I E R G L R							
GGT GTC AGG CAC AAC TCC AGC CTC CTC CAG CAC CAG CAG CAG CAC AGC CTG CGC CAG CTG CTG GTG TTG	730	740	750	760	770	780	790
G V R H N S S L L Q D Q Q H S L R Q L L V L							
TTG CGG CAC CTG GTG CAA GAA AGG GCT AAC GCC TCG GCC TCG GCC TCG GCC TTC ATA ATG GCA GGT GAG CAG	800	810	820	830	840	850	
L R H L V Q E R A N A S A P A F I N A G E Q							
GTG TTC CAG GAC TGT GCA GAG ATC CAG CGC TCT GGG GCC AGT GCC AGT GTC TAC ACC ATC CAG	860	870	880	890	900	910	920
V F Q D C A E I Q R S G A S A S G V Y T I Q							
GTG TCC AAT GCA ACG AAG CCC AGG AAG GTG TTC TGT GAC CTG CAG AGC AGT GCA GGT GTC TAC ACC ATC CAG	930	940	950	960	970	980	990
V S N A T K P R K V F C D L Q S S G R W T							
CTC ATC CAG CGC CGT GAG AAT GGC ACC GTG AAT TTT CAG CGG AAC TGG AAG GAT TAC AAA CAG CGC	1000	1010	1020	1030	1040	1050	
L I Q R R E N G T V N F Q R N W K D Y K Q G							
TTC GGA GAC CCA GCT GGG GAG CAC TGG CTC GGC AAT GAA GTG GTG CAC CAG CTC ACC AGA AGG GCA	1060	1070	1080	1090	1100	1110	1120
F G D P A G E H W L Q N E V H Q L T R R A							
GCC TAC TCT CTG CGT GTG GAG CTG CAA GAC TGG GAA GGC CAC GAG GCC TAT GCC CAG TAC GAA CAT	1130	1140	1150	1160	1170	1180	
A Y S L R V E L Q D W E G H E A Y A Q Y E H							

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1190	TTC	CAC	CTG	GGC	AGT	GAG	AAC	CAG	CTA	TAC	AGG	CTT	TCT	GTG	GTC	GGG	TAC	AGC	GGC	TCA	GCA	GGG	
F	H	L	G	S	E	N	Q	L	Y	R	L	S	V	V	G	Y	S	G	S	A	G		
1260	CGC	CAG	AGC	AGC	CTG	CTG	CTG	CAG	AAC	ACC	AGC	TTT	AGC	ACC	CTT	GAC	TCA	GAC	AAC	GAC	CAC	TGT	
R	Q	S	S	L	V	L	Q	N	T	S	F	S	T	L	D	S	D	N	D	H	C		
1330	CTC	TGC	AAG	TGT	GCC	CAG	GTG	ATG	TCT	GGA	GGG	TGG	TGG	TTT	GAC	GCC	TGT	GCC	CTG	TCA	AAC	CTC	
L	C	K	C	A	Q	V	M	S	G	G	G	W	W	F	D	A	C	G	L	S	N	L	
1390	1400	1410	1420	1430	1440	1450																	
N	G	V	Y	H	A	P	D	N	K	Y	K	M	D	G	I	R	W	H	Y	F			
1460	1470	1480	1490	1500	1510																		
K	G	G	P	S	Y	S	L	R	A	S	R	M	M	I	R	P	L	D	I	*			

Fig.23(Cont ii).

Fig.24.

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10	20	30	40	50	60	70	80	90
ATG ACA GTT TTC CTT TCC TTT GCT TCC CTC GCT GCC ATT CTC ACT CAC ATA GGG TGC AGC ATT CCA GAA AAC AGT GGG								
N T V F L S F A P L A A I L T H I G C S N Q R R S P E N S G>								
100	110	120	130	140	150	160	170	180
AGA AGA TAT AAC CGG ATT CAA CAT CGG CAA TGT GCC TAC ACT TTC ATT CTC CCA GAA CAC GAT GGC AAC TGT CGT GAG AGT AGC GAC								
TCT TCT ATA TTG GCC TAA GTC CCA CCC GTC ACA CGG ATG TGA AAG TAA GAA GGT CTT GTC CTA CGG TTG ACA GCA CTC TCA TGC TGT CTG								
R R Y N R I Q H G Q C A Y T F I L P E H D G N C R E S T T D>								
190	200	210	220	230	240	250	260	270
CAG TAC AAC ACA AAC GCT CTC CAG AGA GAT GCT CCA CAC GTG GAA CCG GAT TTC TCT TCC CAG AAA CTT CAA CAT CTC GAA CAT GTC ATG								
GTC ATG TTG TTG GCA GAC GTC TCT CTA CGA AAG GTC CAC CTC GTC TTA AAG AGA AAG GTC TTT GAA GTC CTT GTC GAC CTT GTC CAC TAC								
Q Y N T N A L Q R D A P H V E P D F S S Q K L Q H L E H V M>								
280	290	300	310	320	330	340	350	360
GAA AAT TAT ACT CAG TGG CTC CAA AAA CTT GAG AAT TAC ATT GTG GAA AAC ATG AAG TGG GAG ATG GCC CAG ATA CAG CAG AAT GCA GTC								
CTT TTA ATA TGA GTC ACC GAC GTC ATT TTT GAA CTC TTA ATG TAA CAC CTT TTG TAC TTC AGC CTC TAC CGG GTC TAT GTC GTC TTA CGT CAA								
E N Y T Q W L Q K N Y I V E N M K S E M A Q I Q Q N A V>								
370	380	390	400	410	420	430	440	450
CAG AAC CAC ACG GCT ACC ATG CTG GAG ATA GGA ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC AGA AAG CTC ACA GAT GTC GAG ACC								
GTC TTG GTG TGC CGA TGG TAC GAC CTC TAT CCT TGG TCG GAG AGA GTC TGA CCT CTC GTC TGG TCT GTC GAC TGT CTA CAA CTC TGG								
Q N H T A T H L E I G T S L L S Q T A E Q T R K L T D V E T>								
460	470	480	490	500	510	520	530	540
CAG GTC CTA AAT CAA ACT TCT CGA CTT GAG ATA CAG CTG CTG GAG AAT TCA TTA TCC ACC TAC AAG CTA GAG AAG CAA CTT CTT CAA CAG								
GTC CAT GAT TTA GTT TGA AGA GCT GAA CTC TAT GTC GAC GTC CTC TTA AGT AAT AGG TGG ATG TGC GAT CTC TTC GAA GTC GTC								
Q V L N Q T S R L E I Q L E N S L E N S T Y K L E K Q L L Q Q>								

Fig.24(Cont i).

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550	560	570	580	590	600	610	620	630
ACA AAT GAA ATC TTG AAG ATC CAT GAA AAA AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA ATG GAA AAA AAC CAC AAG GAA GAG TGG GAC								
TGT TTA CTT TAG AAC TTC TAG GAA CTT CTT AAT AAC CTT GAA TTT TAG AAT CTT TAC CTT CCT TTT GTC TTC CTT CTC AAC CTG	T	N	E	I	L	K	H	
640	650	660	670	680	690	700	710	720
ACC TTA AAG GAA GAG AAC CTT CAA CGC TTG GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTC GAA AAG CAA TTA AAC AGA GCT								
TGG AAT TTC CTT CTC CCT CTC TTG GAA GTT CCG AAC CAA TGA GCA GTC GAT TGT ATA TAT TAG GTC CTC GAC CTT TTC GTC ATT TGG TGT CGA	T	L	K	E	E	N	L	Q
730	740	750	760	770	780	790	800	810
ACC ACC AAC AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTC ATG GAC ACA GTC CAC AAC CTC GTC AAT CTT TGC ACT AAA GAA GGT GTT								
TGG TGG TTG TCA CAG GAA GTC TTC GTC GTT GAC CTC GAC TAC CTC TGT GAA CAG TGA TTT CTT CCA CAA	T	T	N	N	S	V	L	Q
820	830	840	850	860	870	880	890	900
TAA CTA AAG GGA GAA AGA GAG GAA GAG AAC TTT AGA GAC TGT GCT GAA GAA TGT AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC								
ATG GAT TTC CCT CCT TTT CCT CTC CTC CTT CTC AAC TGT AAA TCT CTC ACA CGA CCT CAT AGG TTT AGT CCT GTG TGT TAA GCA TAG ATG	L	L	K	G	R	E	K	P
910	920	930	940	950	960	970	980	990
ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT GGA GGA GGG TGG ACA ATT CAG CGA CGT								
TGC AAT TGT AAG GGA TTA AGA TGT CTT CTC TAG TTC CGG ATG ACA CTG TAC CTT CGA CCT CCT CGG CCC ACC TGT TAA GTC GCT GCA	T	L	T	F	P	N	S	T
1000	1010	1020	1030	1040	1050	1060	1070	1080
GAG GAT GGC AGC GTC GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA TAT TGG CTC GGA ATT GAG								
CTC CTA CCG TGG CAA CTA AAA GTC TCC TGA ACC TTT CCT ATA TTT CCT CCA CCT AAA CCT CCT CCT ATA ACC GAC CCT TTA CTC	E	D	G	S	V	D	F	Q
1090	1100	1110	1120	1130	1140	1150	1160	1170
TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CCT AAA AAC CTC TTT GCA TGT GAA GGG AAT GAG GCT TAC TCA TGG TAT GAA								
AAA CAA AGC GTT GAC TGA TTA GTC GTC GTT GCG ATA AAC GAA TTT TAT GTG GAA ACC CCT CCC TTA CTC CGA ATG AGT AAC ATA CTT	F	V	S	Q	L	T	N	Q

Fig.24(Cont ii).

1180	1190	1200	1210	1220	1230	1240	1250	1260
CAT	TTC	TCG						
TAT	CTC	TCA	AGT	GAA	GAA	GAA	GAA	GAA
GTA	AAG	ATA	GAG	AGT	TCA	CTT	CTT	CTT
H	F	Y	L	S	E	L	N	Y
1270	1280	1290	1300	1310	1320	1330	1340	1350
CCA	GGG	AAT	GAT	TTT	AGC	ACA	AGG	GAT
GGT	CCT	TTA	CTA	AAA	TGG	GAA	GAC	AAA
P	G	N	D	F	S	T	K	D
1360	1370	1380	1390	1400	1410	1420	1430	1440
GCA	TGT	GCT	TCC	AAC	TTC	AAC	GGG	ATT
CGT	ACA	CCA	GGG	AGG	TTC	AAA	TGG	AAA
A	C	G	G	P	S	N	T	T
1450	1460	1470	1480	1490				
GGC	TCA	GGC	TAT	TGC	CTC	AAG	GGC	ACA
CCG	AGT	CCG	ATA	AGC	GAG	TTC	CGG	TGT
G	S	G	Y	S	L	K	A	T

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10. 20. 30. 40. 50. 60. 70. 80. 90. *
 ATG TGG CAG ATT GTT TTC ACT CTG AGC TGT GAT CTT GTC TTG GCC GCA CCC TAT AAC AAC TTT CGG AAG AGC ATG GAC AGC ATA GGA
 TAC ACC GTC TAA CAA GAC TCG ACA CTA GAA CAG AAC CGG CGT CCG ATA TTG TTG AAA GCC TTC TCG TAC CGT TAT CCT
 H W Q I V F T L S C D L V A Y N N F R K S M D S I G>
 100. 110. 120. 130. 140. 150. 160. 170. *
 AAG AAG CAA TAT CAG GTC CAG CAT GGG TCC TGC AGC TAC ACT TTC CTC CTC GCA GAG ATG GAC AAC TGC CGC TCT TCC TCC AGC CCC TAC
 TTC TTC GTT ATA GTC CAG GTC GTC GTC CCC AGG ACG TCG ATG TGA AAG GAG GAC GGT CTC TAC CTG TTG AGG GCG AGA AGG AGG TCG GGG ATG
 K K Q Y Q V S C S Y T F L L P E M D N C R S S P Y>
 190. 200. 210. 220. 230. 240. 250. 260. 270. *
 GTG TCC ATT GCT GTG CAG AGG GAC GGG CCG CTC GAA TAC GAT GAC TCG GTG CAG AGG CTC CAA GTG CTG GAG AAC ATC ATG GAA AAC AAC
 CAC AGG TTA CGA CAC GTC TCC CGC CGC GAG CTT ATG CTA CGT AGC CAC GTC TCC GAC GTT CAC GAC CTC TTG TAG TAC CTT TTG TTG
 V S N A V Q R D A P L E Y D P S V R L Q V L E N I H E N N>
 280. 290. 300. 310. 320. 330. 340. 350. 360. *
 ACT CAG TGG CTA ATG AAG CTT GAG AAT TAT ATC CAG GAC AAC ATG AAG AAA GAA ATG GTA GAG ATA CAG AAT GCA GTC CAA GTC AAC CAG
 TGA GTC ACC GAT TAC TTC GAA GTC TTA ATA TAG GTC CTG TTG TAC TTC TTT CTT TAC CAT CTC TAT GTC GTC TTA CGT CAT GTC TTG GTC
 T Q W L H K L E N Y I Q D N H K R E M V E I Q Q N A V Q N Q>
 370. 380. 390. 400. 410. 420. 430. 440. 450. *
 ACG GCT GTG ATG ATA GAA ATT GGG ACA AAC CTC TTG AAC GAA ACA GCT GAG CAA AGC CGG AAG TTA ACT GAT GTG GAA GCC CAA GTC TTA
 TGC CGA CAC TAC TAT CTT TAT CCC TGT TTG GTC AAC TTG GTT TGT CGA CTC GTT TGC GCC TTG ATG TGA CTA CAC CTC CGG GTT CAT ATT
 T A V M I E I Q T N L L N Q T A E Q T R K L T D V E A Q V L>
 460. 470. 480. 490. 500. 510. 520. 530. 540. *
 ATT CAG ACC ACC AGA ATT GAA CTT CAG CTC TTG GAA CAC TCC CTC TCG ACA AAC AAA TTG GAA AAA CAG ATT TTG GAC CAG ACC AGT GAA
 TTA GTC TGG TGC TCT GAA CTT GAA GTC GAG AAC CTT GTG AGG GAG AGC TGT TTG AAC CTT TTT GTC TAA AAC CTC GTC TGG TCA CTT
 N Q T T R L E L Q L L E H S L S T N K L E K Q I L D Q T S E>

Fig.25.

Fig.25(Cont i).

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550	560	570	580	590	600	610	620	630
ATA AAC AAA TTG CAA GAT AAG AAC AGT TCC CTA GAA AAG GTG CTA GCT ATG GAA GAC AAG CAC ATC CAA CTA CAG TCA ATA AAA	TAT TTG TTT AAC GTT CTA TTC TGT TCA AAG GAT CTT TTC CAC GAT CGA TAC CTT CTC GTG TAG GTT GAT GTC AGT TAT TTT	I N K L Q D K N S P L E K K V L A M E D K H I I Q L Q S I K >						
640	650	660	670	680	690	700	710	720
GAA GAG AAA GAT CAG CTA CAG GTG TTA GTA TCC AAG CAA AAT TCC ATC ATT GAA GAA CTA GAA AAA AAA ATA GTG ACT GCC AGC GTG AAT	CCT CTC TTT CTA GTC GAT GTC CAC AAT CAT AGG TTC GTT TTA AGG TAG TAA CTT CTT GAT CTT TTT TAT CAC TGA CGG TGC CAC TTA	E E K D Q L V S K Q N S I I E E L E K K I V T A T V N >						
730	740	750	760	770	780	790	800	810
AAT TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC ATG GAG ACA GTT AAT AAC TTA CTC ACT ATG ATG TCC ACA TCA AAC TCA GCT AGC	TTA AGT CAA GAA GTT TTC GTC GTT GTC CTA GAG TAC CTC TGT CAA TTA TGG AAT GAC TGA TAC TAC AGG TGT AGT CGA TTC CTG	N S V L Q K Q O H D L M E T V N N L T M H S T M H S T S N S A K D >						
820	830	840	850	860	870	880	890	900
CCC ACT GTT CCT AAA GAA GAA CAA ATC ACC TTC AGA GAC GAT TGT GCA GAT TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT	GGA TGA CAA CGA TTT CCT GTT TAG TCG AAG TCT CTG ACA CGT CTA CAT ATA GTT CGA CCA AAA TTA TTT TCA CCT ATG TGA TAA	P T V A K E E Q I S F R D C A D V Y Q A G F N K S G I Y T I >						
910	920	930	940	950	960	970	980	990
TAT ATT AAT ATT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT	ATA TAA TTA TAC CGT CTT CGG TTT TGC CAC AAA AGC TTA TAC CTA CGG TTA CCC CCT CCA ACC TGA CAT TAT GTT GCA CTT CTA	Y I N N H P E P K V F C N M D V N G G W T I Q H R E D >						
1000	1010	1020	1030	1040	1050	1060	1070	1080
GGA AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA ATT ATG GGT TTT GGA AAT CCC TCC GGT GAA ATT TGG CTG GGG AAT GAG TTT ATT	CCT TCA GAT CTA AAG GTT TCT CGG ACC TTC CTT ATA TTT TAC CCA AAA CCT TTA GGG AGG CCA CCT ATA ACC GAC CCC TTA CTC AAA TAA	G S L D F Q R G W K E Y K H G F G N P S G E Y W L G N E F I >						
1090	1100	1110	1120	1130	1140	1150	1160	1170
TTT GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA AAC CGA AAC CGA GGC TAT TCA CAG TAT GAC AGA TTC	AAA CGG TAA TGG TCA GTC TCC GTC ATG TAC GAT TCT TTA CTC AAT TAC CGT ACC CTT CCC TTG GCT CGG ATA AGT GTC ATA CTG TCT AGG	F A I T S Q R Q Y M L R I E L H D W E G N R A Y S Q Y D R P >						

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1180 1190 1200 1210 1220 1230 1240 1250 1260 *
 CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT
 GTG TAT CCT TTA CTT TTC GTT ATA TCC AAC ATA AAT TTT CCA GTG TGA CCC TGT CGT CCT TTT GTC TCG TCG GAC TAG AAT GTG CCA
 H I G N E K Q N Y R L Y L K G H T G T A G R Q S L I L H G>
 1270 1280 1290 1300 1310 1320 1330 1340 1350 *
 GCT GAT TTC AGC ACT AAA GAT GCT GAT ATT GAC AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT
 CGA CTA AAG TCG TGA TTT CTA CGA CTA TTA CTC TTG ACA TAC ACG TTT ACA TAC CCT CCT ACC ACC AAA CTA CGA ACA
 A D F S T K D A D N C M C R C A L M L T G W W F D A C>
 1360 1370 1380 1390 1400 1410 1420 1430 1440 *
 GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC
 CCG GGG AGG TTA GAT TTA CCT TAC AAG ATA TGA CGC CCT GTT TGC GTA CCT TTT GAC TTA CCC TAT TPC ACC GTC ATG AGG TTT CCC GGG
 G P S N L N G M F Y T A G Q N H G K L N G I R W H Y F K G P>
 1450 1460 1470 1480 1490 *
 AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT TGA
 TCA ATG AGG AAT GCA AGG TGT TGA TAC TAC TAA GCT GGA AAT CTA AAA ACT
 S Y S L R S T T M M I R P L D F *>

Fig.25(Cont ii).

Fig.26.

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10	20	30	40	50	60	70	80	90																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
ATG	ACA	GTT	TTC	CCT	TCC	TTC	CTG	ACT	CAC	ATA	GGG	TGG	AGC	AAAT	CAG	CGG	CGA	AGT	CCA	AAC	AGT	GGG																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
TAC	TGT	CAA	AAG	GAA	AGG	AAA	CGA	AGG	CGG	TAA	GAC	GCA	TGG	TAA	GTC	GCG	GCT	TCA	GGT	CTT	TTC	CCC																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
H	V	T	F	L	S	P	A	F	L	A	A	I	H	I	G	C	S	P	R	R	S	E	N	S	G>																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
AGA	AGA	TAT	AAC	CGG	ATT	CAA	TGT	GCC	TAC	ACT	TTC	ATT	CTT	CCA	GAA	CAC	GAT	GGC	AAC	TGT	CGT	GAG	AGT	ACG	ACA	GAC	36/41																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
TCT	TCT	ATA	TTC	GCC	TAA	CGG	TAA	GTC	TCA	GGT	CTT	GTC	CTA	CCG	TTC	ACA	GCA	CTC	TCA	TGC	TGT	TCT	R	R	Y	N	R	I	Q	H	G	Q	C	A	Y	T	F	I	L	P	E	H	D	G	N	C	R	E	S	T	T	D>																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700	710	720	730	740	750	760	770	780	790	800	810	820	830	840	850	860	870	880	890	900	910	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690	1700	1710	1720	1730	1740	1750	1760	1770	1780	1790	1800	1810	1820	1830	1840	1850	1860	1870	1880	1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990	2000	2010	2020	2030	2040	2050	2060	2070	2080	2090	2100	2110	2120	2130	2140	2150	2160	2170	2180	2190	2200	2210	2220	2230	2240	2250	2260	2270	2280	2290	2300	2310	2320	2330	2340	2350	2360	2370	2380	2390	2400	2410	2420	2430	2440	2450	2460	2470	2480	2490	2500	2510	2520	2530	2540	2550	2560	2570	2580	2590	2600	2610	2620	2630	2640	2650	2660	2670	2680	2690	2700	2710	2720	2730	2740	2750	2760	2770	2780	2790	2800	2810	2820	2830	2840	2850	2860	2870	2880	2890	2900	2910	2920	2930	2940	2950	2960	2970	2980	2990	3000	3010	3020	3030	3040	3050	3060	3070	3080	3090	3100	3110	3120	3130	3140	3150	3160	3170	3180	3190	3200	3210	3220	3230	3240	3250	3260	3270	3280	3290	3300	3310	3320	3330	3340	3350	3360	3370	3380	3390	3400	3410	3420	3430	3440	3450	3460	3470	3480	3490	3500	3510	3520	3530	3540	3550	3560	3570	3580	3590	3600	3610	3620	3630	3640	3650	3660	3670	3680	3690	3700	3710	3720	3730	3740	3750	3760	3770	3780	3790	3800	3810	3820	3830	3840	3850	3860	3870	3880	3890	3900	3910	3920	3930	3940	3950	3960	3970	3980	3990	4000	4010	4020	4030	4040	4050	4060	4070	4080	4090	4100	4110	4120	4130	4140	4150	4160	4170	4180	4190	4200	4210	4220	4230	4240	4250	4260	4270	4280	4290	4300	4310	4320	4330	4340	4350	4360	4370	4380	4390	4400	4410	4420	4430	4440	4450	4460	4470	4480	4490	4500	4510	4520	4530	4540	4550	4560	4570	4580	4590	4600	4610	4620	4630	4640	4650	4660	4670	4680	4690	4700	4710	4720	4730	4740	4750	4760	4770	4780	4790	4800	4810	4820	4830	4840	4850	4860	4870	4880	4890	4900	4910	4920	4930	4940	4950	4960	4970	4980	4990	5000	5010	5020	5030	5040	5050	5060	5070	5080	5090	5100	5110	5120	5130	5140	5150	5160	5170	5180	5190	5200	5210	5220	5230	5240	5250	5260	5270	5280	5290	5300	5310	5320	5330	5340	5350	5360	5370	5380	5390	5400	5410	5420	5430	5440	5450	5460	5470	5480	5490	5500	5510	5520	5530	5540	5550	5560	5570	5580	5590	5600	5610	5620	5630	5640	5650	5660	5670	5680	5690	5700	5710	5720	5730	5740	5750	5760	5770	5780	5790	5800	5810	5820	5830	5840	5850	5860	5870	5880	5890	5900	5910	5920	5930	5940	5950	5960	5970	5980	5990	6000	6010	6020	6030	6040	6050	6060	6070	6080	6090	6100	6110	6120	6130	6140	6150	6160	6170	6180	6190	6200	6210	6220	6230	6240	6250	6260	6270	6280	6290	6300	6310	6320	6330	6340	6350	6360	6370	6380	6390	6400	6410	6420	6430	6440	6450	6460	6470	6480	6490	6500	6510	6520	6530	6540	6550	6560	6570	6580	6590	6600	6610	6620	6630	6640	6650	6660	6670	6680	6690	6700	6710	6720	6730	6740	6750	6760	6770	6780	6790	6800	6810	6820	6830	6840	6850	6860	6870	6880	6890	6900	6910	6920	6930	6940	6950	6960	6970	6980	6990	7000	7010	7020	7030	7040	7050	7060	7070	7080	7090	7100	7110	7120	7130	7140	7150	7160	7170	7180	7190	7200	7210	7220	7230	7240	7250	7260	7270	7280	7290	7300	7310	7320	7330	7340	7350	7360	7370	7380	7390	7400	7410	7420	7430	7440	7450	7460	7470	7480	7490	7500	7510	7520	7530	7540	7550	7560	7570	7580	7590	7600	7610	7620	7630	7640	7650	7660	7670	7680	7690	7700	7710	7720	7730	7740	7750	7760	7770	7780	7790	7800	7810	7820	7830	7840	7850	7860	7870	7880	7890	7900	7910	7920	7930	7940	7950	7960	7970	7980	7990	8000	8010	8020	8030	8040	8050	8060	8070	8080	8090	8100	8110	8120	8130	8140	8150	8160	8170	8180	8190	8200	8210	8220	8230	8240	8250	8260	8270	8280	8290	8300	8310	8320	8330	8340	8350	8360	8370	8380	8390	8400	8410	8420	8430	8440	8450	8460	8470	8480	8490	8500	8510	8520	8530	8540	8550	8560	8570	8580	8590	8600	8610	8620	8630	8640	8650	8660	8670	8680	8690	8700	8710	8720	8730	8740	8750	8760	8770	8780	8790	8800	8810	8820	8830	8840	8850	8860	8870	8880	8890	8900	8910	8920	8930	8940	8950	8960	8970	8980	8990	9000	9010	9020	9030	9040	9050	9060	9070	9080	9090	9100	9110	9120	9130	9140	9150	9160	9170	9180	9190	9200	9210	9220	9230	9240	9250	9260	9270	9280	9290	9300	9310	9320	9330	9340	9350	9360	9370	9380	9390	9400	9410	9420	9430	9440	9450	9460	9470	9480	9490	9500	9510	9520	9530	9540	9550	9560	9570	9580	9590	9600	9610	9620	9630	9640	9650	9660	9670	9680	9690	9700	9710	9720	9730	9740	9750	9760	9770	9780	9790	9800	9810	9820	9830	9840	9850	9860	9870	9880	9890	9900	9910	9920	9930	9940	9950	9960	9970	9980	9990	10000

Fig.26(Cont i).

550. 560. 570. 580. 590. 600. 610. 620. 630. *

ACC AGT GAA ATA AAC AAA TGG CAA GAT AAC AGT TTC CTA GAA AAG AAG GTG CTA CCT ATG GAA GAC AAG CAC ATC CAA CTA CAG TGG TCA CTT TAT TGG TTT AAC GAT CTT CTC TCA AAG GAT CCT TTC CAC GAT CGA TAC CTT CTG TTG TAG TAG GTT GAT GTC T S E I N K L Q D K N S F L E K V L A H E D K H I I Q L Q>

640. 650. 660. 670. 680. 690. 700. 710. 720. *

TCA ATA AAA GAA GAG AAA GAT CAG CTA CAG GTG TTA GTC TCC AAG CAA AAT TCC ATC ATT GAA GAA CTA GAA AAA AAA ATA GTG ACT GCC AGT TAT TTT CTC TTT CTA GTC GAT GTC CAC AAC ATG TTC GTT TTA AGG TAG TAA CTT GAT CTT TTT TAT CTC TGA CGG S I K E E K D Q L Q V L S K V L S N S I I E E L E K K I V T A>

730. 740. 750. 760. 770. 780. 790. 800. 810. *

ACG GTG AAT AAC TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC ATG GAG ACA GTT AAT AAC TTA CTG ACT ATG ATG TCC ACA TCA AAC TCA TGC CAC TTA TTA AGT GAA GTT TTC GTC GTC CAA CTA GAG TAC CTC TGT CAA TTA TTG AAT GAC TGA TAC TAC AGG TGT AGT TTG AGT T V N N S V L Q K Q H D L N E T V N N L L T M M S T S N S>

820. 830. 840. 850. 860. 870. 880. 890. 900. *

GCT AAG GAC CCC ACT GTT CCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCT GAA GCA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC CGA TTC CTC CGG TGA CAA CGA TTT CTT GTC GTT TAG TCG AAG TGT CTC CGA CCT CAT AAG TTT AGT CCT GTC TGT TTA CCG TAG A K D P T V A K E Q I S F R D C A E V F K S G H T T N G I>

910. 920. 930. 940. 950. 960. 970. 980. 990. *

TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA CCT GGA CGA CGG TGG ACA ATT ATT CAG CGA ATG TGC AAT TGT AAG GGA TTA AGA TGT CTT CTC TGT ACA CGG ATG ACA CGT TAC CCT CGA CCT CCT CGG CCC ACC TGT TAA GTC GCT Y T L T F P N S T E E I K A Y C D H E A G G W T I I Q R>

1000. 1010. 1020. 1030. 1040. 1050. 1060. 1070. 1080. *

CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG CGG TTT CCT AAC CCT TCA GGA TAT TGG CTG GGA AAT GCA CTC CTA CGG TCG CAA CTA AAA GTC TCC ACC TTT CTT CTC AAC CCT AAA CCA TTG GGA AGT CCT CTT ATA ACC GAC CCT TTA R E D G S V D F Q R T W K E Y K V G F G N P S G E Y W L G N>

Fig.26(Cont ii).

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1090	1100	1110	1120	1130	1140	1150	1160	1170
*	*	*	*	*	*	*	*	*
GAG TTT GTT TCG CAA CTC ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT								
CTC AAA CAA AGC GTT GAC TGA TTA GTC GTT GGG ATA CAC GAA TTT TAT GTG GAA TTT CTC ACC CTT CCC TTA CTC CGA ATG AGT AAC ATA								
E F V S Q L T N Q R Y V L K I H L K D W E G N E A Y S L Y>								
1180	1190	1200	1210	1220	1230	1240	1250	1260
*	*	*	*	*	*	*	*	*
GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GGC GGC AAA ATA AGC AGC ATC AGC								
CCT GTA AGG ATA GAG AGT TCA CTT CCT GAG TTA ATA TCC TAA GTG GAA TTT CCT GAA TGT CCC TGT CGG CGG TTT TAT TCG TCG TAG TCG								
E H F Y L S S E E L N Y R I H L K G L T A G K I S S I S>								
1270	1280	1290	1300	1310	1320	1330	1340	1350
*	*	*	*	*	*	*	*	*
CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT								
GTT GGT CCT TTA CTA AAA TCG TGT TTC CTA CCT CCT TCG TTT ACA TAA AGC TTT ACA AGT GTT TAC GAT TGT CCT CGG ACC ACC AAA								
Q P G N D F S T K D G N D K C I C K C S Q H L T G G W W F>								
1360	1370	1380	1390	1400	1410	1420	1430	1440
*	*	*	*	*	*	*	*	*
GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG								
CTA CGT ACN CCA GGA AGG TGG AAC TTG CCT TAC ATG ATA GGT GTC TCC GTC TGT TTA TTC AAG TTG CGG TAA TTT ACC ATG ATG ACC								
D A C G P S N L N G M Y Y P Q R Q N T N K F N G I K W Y Y W>								
1450	1460	1470	1480	1490	1500			
*	*	*	*	*	*			
AAA GGC TCA GGC TAT TCG CTC AAG GGC ACA ACC ATG ATG ATC CGA CCA GCA GAT TTC TAA								
TTT CCG AGT CCG ATA AGC GAG TTC CGG TGT TGG TAC TAC TAG GCT GGT CGT CTA AAG ATT								
K G S G Y S L K A T T M H I R P A D F >								

Fig.27.

10 20 30 40 50 60 70 80 90
 * * * * * * * * *
 ATG TGG CAG ATT GTT TTC ACT CTG AGC TGT GAT CTT GTC TTG GCC GCC TAT AAC AAC TTT CGG AAG AGC ATG GAC AGC ATA GGA
 TAC ACC GTC TAA CAA AAG AAA TGA GAC TCG ACA CTA GAA CAG AAC CGG CTT CGG ATA TTT TTG AAA GCC TTC TCG TAC CTC TCG TAT CCT
 H W Q I V F P T L S C D L V A A Y N N F R K S M D S I G>
 100 110 120 130 140 150 160 170 180
 * * * * * * * * *
 AAG AAG CAA TAT CAG CAG CAT CGG TGC AGC TAC ACT TTC CTC CTG CCA GAG ATG GAC AAC TGC CGG TCT TCC AGC CCC TAC
 TTC GTC ATT GTC CAG GTC GTC GCA CCC AGG AGG TGC ATG TGA AAG GAG GAC GGT CTC TAC CTG TTG AGC CGG AGA AGG TCG GGG ATG
 K K Q Y Q V Q H G S C S Y T F L P E H D N C R S S P Y>
 190 200 210 220 230 240 250 260 270
 * * * * * * * * *
 GTG TCC AAT GCT GTG CAG AGG GAC GCA CGG CTC GAA TAC GAT TTC TCT TCC CAG AAA CTT CAA CAT CTG GAA CAT GTG ATG GAA AAT TAT 39/41
 CAC AGG TTA CGA CAC GTC TCC CTC CGC GGC GAG CTT ATG CTA AAG AGA AGG GTC TTT GAA GTT GTC GAC CTT GTC CAC TAC CTT TTA ATA
 V S N A V Q R D A P L E Y D F S S Q K L Q H L E H V N E N Y>
 280 290 300 310 320 330 340 350 360
 * * * * * * * * *
 ACT CAG TGG CGT CAA AAA CTT GAG AAT TAC ATT GTG GAA AAC ATG AAG TCG GAG ATG GCC CAG ATA CAG CAG AAT GCA GTT CAG AAC CAC
 TGA GTC ACC GAC GTT TTT GAA CTC TTA ATG TAA CAC CTC TTG TAC TTC AGC CTC TAC CGG GTC TAT GTC GTC TTA CGT CAA GTC TTT GTG
 T Q W L Q K L E N Y I V E N H A Q I Q N A V Q N H>
 370 380 390 400 410 420 430 440 450
 * * * * * * * * *
 ACG GCT ACC ATG CTC GAG ATA GGA ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC AGA AAG CTG ACA GAT GTT GAG ACC CAG GTC CTA
 TGC CGA TGG TAC GAC CTC TAT CCT GAG GTC GAG AGA GTC TGA CGT CTC GTC TGT TTC GAC TGT CTA CAA CTC TGG GTC CAT GAT
 T A T H L E I Q T S L L S Q T A E Q T R K L T D V E T Q V L>
 460 470 480 490 500 510 520 530 540
 * * * * * * * * *
 AAT CAA ACT TCT CGA CTT GAG ATA CAG CTG CTG GAG AAT TCA TTA TCC ACC TAC AAG CTA GAG AAG CAA CTT CCA CAG ACA AAT GAA
 TTA GTT TGA AGA GCT GAA CTC TAT GTC GAC GAC CTC TTA AGT ATT AGG TGG ATG TTC GAT CTC TTC GTT GAA GAA GTT GTC TGT TTA CTT
 N Q T S R L E I Q L L E N S T Y R K Q L L Q Q T N E>

Fig.27(Cont i)

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550	560	570	580	590	600	610	620	630
ATC TTG AAG ATC CAT GAA AAA AAC AGT TTA GAA CAT AAA ATC TTA GAA ATG GAA GGA AAA CAC AAG GAA GAG TTG GAC ACC TTA AAG								
TAG AAC TTC TAG GTA CTT TTT TTG TCA ATT ATT CTT GTA TTT TAG ATT CTT TAC ATT CCT CTT CCT CTC AAC CTC TGG ATT TTC	I	L	K	H	E	N	S	L
I L K H E N S L								
640	650	660	670	680	690	700	710	720
GAA GAG AAA GAG AAC CTT CAA GGC TTG GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTG GAA MAG CAA TTA AAC AGA GCT ACC ACC AAC								
CTT CTC TTT CTC TTG GAA GTT CCG AAC CAA TGA GCA GTT TGT ATA TAT TAG GTC CTC GAC CTT TTC GTT ATT TGG TCT CGA TGG TTG	E	E	K	E	N	L	Q	G
E E K E N L Q G L								
730	740	750	760	770	780	790	800	810
AAC AGT GTC CTT CAG CAA CTG GAA GTC CTC GTC ATG GAC CAC AAC GTC CAC TAC CTC TGT GTC GAG GTG GAA CAG TTA GAA AGC TGA ATT CTT CCT GCT ACT AAA GAA GGT GTT TTA CTA AAG								
TTG TCA CAG GAA GTC TTC GTC GTT GAC CTC GAC TAC CTC TGT GTC GAG GTG GAA CAG TTA GAA AGC TGA ATT CTT CCT CCA AAA ATT GAT GTC	N	S	V	L	Q	K	Q	L
N S V L Q K Q L								
820	830	840	850	860	870	880	890	900
GGA GGA AAA AGA GAG AAA CCA TTT AGA GAC TGT GCA GAT GTC TAT CAA GCT GGT ATT ATT AAA AGT GGA ATC TAC ACT ATT ATT								
CCT CCT TTT TCT CTC CTT CTC TTT GGT AAA TCA TCT CGT ACA CGT CTC ATT	G	G	K	R	E	E	K	P
G G K R E E K P R D C A D V Y Q A G F N K S G I Y T I Y >								
910	920	930	940	950	960	970	980	990
ATT ATT ATT ATG CCA GAA CCC AAA AAG GTG TTT TGC ATT ATT GAT GTC ATT GGT GAA ATT GGG GGA GGT TGG ACT GTC ATT CAA CAT CGT GAA GAT GGA								
TAA TTA TTA TAC GGT CTT GGG ATT	I	N	N	H	P	E	P	K
I N N H P E P K V F C N M D V N G G W T V I Q H R E D G >								
1000	1010	1020	1030	1040	1050	1060	1070	1080
AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT ATT GGT TTT GGA ATT CCC TCC GGT GAA TAT GAG TGG CTG GGG ATT ATT ATT								
TCA GAT CTA AAG GTT TCT CTC ACC TTC CTC ATT	S	L	D	F	Q	R	G	W
S L D F Q R G W K E Y K M G F N P S G E Y W L G N E F I F >								

Fig.27(Cont ii)

1090 1100 1110 1120 1130 1140 1150 1160 1170 *
 GCC ATT ACC AGT CAG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC
 CGG TAA TGG TCA GTC TCC GTC ATG TAC GAT TCT TAA CTC ATT TAC CTG ACC CTT CCC TTG CCT CGG ATA AGT GTC ATA CTG TCT AAG GTC
 A I T S Q R Q Y H L R I E L M D W E G N R A Y S Q Y D R F H >
 1180 * 1190 1200 1210 1220 1230 1240 1250 1260 *
 ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGG CTC ATC TTA CAC GGT GCT
 TAT CCT TTA CTT TTC GTC ATA TCC AAC ATA ATT TTT CCA GTG TGA CCC TGT CGT CCT TTT GTC TCG TCG GAC TGG AAT GTG CCA CGA
 I G N E K Q N Y R L Y L K G H T G T A G K Q S S L I L H G A >
 1270 * 1280 1290 1300 1310 1320 1330 1340 1350 *
 GAT TTC AGC ACT AAA GAT GCT GAT ATT GAC AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA TGG TGG TTT GAT GCT TGT TGC
 CTA AAG TCG TGA TTT CTA CGA CTA TTA CGT TTG ACA TAC AGC ATT ACA CGG GAG TAC ATT CCT CCT ACC ACC AAA CTA CGA ACA CGG
 D F S T K D A D N C H C K C A L M L T G G W W F D A C G >
 1360 * 1370 1380 1390 1400 1410 1420 1430 1440 *
 CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT GGA AAA CTG ATT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC AGT
 GGG AGG TTA GAT TTA CCT TAC AAG ATA TGA CGC CCT GTC ATT GAC TTA CCC TAT TTC ACC GTC ATG AGG TTT CCC GGG TCA
 P S N L N G M F Y T A G Q N H G K L N G I K W H Y F K G P S >
 1450 * 1460 1470 1480 *
 TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT TGA
 ATG AGG AAT GCA AGG TGT TGA TAC TAA GCT GGA AAT CTA AAA ACT
 Y S L R S T T H I R P L D F >

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/13557

A. CLASSIFICATION OF SUBJECT MATTER				
IPC 6	C12N15/12	C07K14/515	C07K14/71	C07K16/22
	C07K19/00	A61K38/17	A61K38/18	A61K39/395
	A61K51/08	C12Q1/48	C12Q1/68	G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	<p>WO 96 11269 A (REGENERON PHARMA ;DAVIS SAMUEL (US); BRUNO JOANNE (US); GOLDFARB M) 18 April 1996 see abstract</p> <p>see page 7, line 1 - page 9, line 3 see page 33, line 20 - page 36, line 17; example 2 see page 42, line 17 - page 52, line 20; examples 6-9 see page 54, line 6 - page 58, line 18; examples 11,12 see page 68 - page 76; claims ---</p> <p style="text-align: right;">-/-</p>	<p>30-33, 38,40, 48-50 1-4, 11-20, 22-29, 34-37, 39,41-47</p>

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

Date of mailing of the international search report

12 November 1997

11/12/1997

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/13557

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A01K67/027 C12N1/19 C12N1/21 C12N5/08 C12N5/10
 C12N5/22 // (C12N1/19, C12R1:645), (C12N1/21, C12R1:01),
 A61K121:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 96 31598 A (REGENERON PHARMA ;DAVIS SAMUEL (US); BRUNO JOANNE (US); GOLDFARB M) 10 October 1996</p> <p>see page 39 - page 41; example 2 see page 59, line 21 - page 64, line 2; examples 11,12 see page 68, line 15 - page 71, line 25; examples 18-20 see page 75 - page 84; claims see figure 17</p> <p>---</p> <p style="text-align: center;">-/-</p>	9,14,19, 22-33, 38,40, 48-50

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Date of the actual completion of the international search

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/13557

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X 3	MAISONPIERRE P.C. ET AL.: "ANGIOPOETIN-2, A NATURAL ANTAGONIST FOR TIE-2 THAT DISRUPTS IN VIVO ANGIOGENESIS" SCIENCE, vol. 277, 4 July 1997, pages 55-60, XP002046280 note 15 see page 60, left-hand column -----	9, 19, 22-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/13557

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9611269 A	18-04-96	US	5643755 A	01-07-97
		US	5521073 A	28-05-96
		US	5650490 A	22-07-97
		AU	4129596 A	02-05-96
		EP	0784683 A	23-07-97
		FI	971406 A	04-06-97
		NO	971557 A	06-06-97
		PL	319586 A	18-08-97
		AU	5387196 A	23-10-96
		WO	9631598 A	10-10-96
<hr/>				
WO 9631598 A	10-10-96	AU	4129596 A	02-05-96
		AU	5387196 A	23-10-96
		EP	0784683 A	23-07-97
		FI	971406 A	04-06-97
		NO	971557 A	06-06-97
		PL	319586 A	18-08-97
		WO	9611269 A	18-04-96
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